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(54) Title: FOUR NOVEL RECEPTORS OF THE TGF-β RECEPTOR FAMILY

(57) Abstract

Isolated DNAs (e.g., cDNAs or genomic fragments) encoding MIS receptors, inhibin receptors, bone morphogenic protein receptors, or other novel members of the TGF-β family of receptors, or soluble, ligand-binding fragments thereof; vectors or cells which contain such DNAs; and substantially pure polypeptides encoded by such DNAs, whether produced by expression of the isolated DNAs, by isolation from natural sources, or by chemical synthesis.

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FOUR NOVEL RECEPTORS OF THE TGF-β RECEPTOR FAMILY

Background of the Invention

The field of the invention is mammalian receptor proteins, and nucleic acids encoding same.

Müllerian Inhibiting Substance (MIS) plays a critical role in normal sexual dimorphism as one of the early manifestations of the SRY genetic switch (Gubbay et al., Nature 346:245-250, 1990; Sinclear et al., Nature 346:240-244, 1990; Berta et al., Nature 348:448-350, 1990; Haqq et al., Proc. Natl. Acad. Sci. USA 90:1097-1101, 1993). MIS subsequently causes regression of the Müllerian duct, inhibition of aromatase activity which leads to increased synthesis of testosterone, and probably morphological differentiation of the sex cords as seminiferous tubules, thus assuring the male phenotype. Jost's seminal observations in the late 1940s first defined a "Müllerian Inhibitor" responsible for regression of the Müllerian ducts in the male mammalian embryo (Jost, Arch. Anat. Micro. Morphol. Exp. 36:271-315, 1947). MIS was found to be a 140 kDa protein produced by the Sertoli cell (Blanchard and Josso, Pediatr. Res. 8:968-971, 1974); it was subsequently purified to homogeneity (Budzik et al., Cell 21:909-915, 1980, Cell 34:307-314, 1983; Picard et al., Mol. Cell. Endocrinol. 34:23, 1984), using the bioassay of Müllerian duct regression devised by Picon (Arch. Anat. Microsc. Morphol. Exp. 58:1-19, 1969) as a monitor. The bovine and human genes were cloned (Cate et al., Cell 45:685-698, 1986a) and subsequently expressed and produced in mammalian cell cultures (Cate et al., Cold Spring Harbor Symposium 51:641-647, 1986b; Epstein et al., In Vitro Cellular and Developmental Biol. 25:213-216, 1989); more recently, the rat (Haqq et al., Genomics 12:665-9, 1992) and mouse (Munsterberg and Lovell-Badge, Development

13:613-624, 1991) genes have also been cloned. Overexpression of MIS in transgenic female mice caused regression of Müllerian ducts and seminiferous tubular differentiation (Behringer et al., Nature 345:167-70, 1991). Several patients with Retained Müllerian Duct Syndrome were found to have point mutations in the MIS gene (Knebelman et al., Proc. Natl. Acad. Sci. 88:3767-3771, 1991), which has been localized to the short arm of chromosome 19 (Cohen-Hagenaur et al., Cytogenet. Cell. Genet. 44:2-6, 1987). In mice, the MIS gene is located on chromosome 10 (King et al., Genomics 11:273-283, 1991).

MIS is a member of the large TGF-β family, which includes, besides TGF-β (Derynck et al., Nature 316:701-5, 1985), activin (Ling et al., Nature 321:779-82, 1986; Vale et al., Nature 321:776-779, 1986); inhibin (Mason et al., Nature 318:659-63, 1985); decapentaplegia complex (Padgett et al., Nature 325:81-4, 1987); Vg-1 (Weeks and Melton, Cell 51:861-7, 1987); and bone morphogenesis factors (Wozney et al., Science 242:1528-34, 1988). A common feature of some members of this gene family is that latent precursor can be activated by plasmin cleavage and release of 25 kDa carboxyl terminal dimers.

Although originally defined and named by its ability to cause regression of the Müllerian duct, other functions have emerged for MIS. Its localization to the preantral and smaller antral follicles by immunocytochemical techniques (Takahashi et al., Biol. Reprod. 35:447-53, 1986a; Bezard et al., J. Reprod. Fertil. 80-509-16, 1987; Ueno et al., Endocrinol. 125:1060-1066, 1989a; Ueno et al., Endocrinology 124:1000-1006, 1989b) and its ability to inhibit germinal vesicle breakdown (Takahashi et al., Mol-Cell-Endocrinol. 47:225-34, 1986b; Ueno et al., Endocrinology 123:1652-1659, 1988) led to the hypothesis that it was involved in

meiotic inhibition in the ovary. Developmental and experimental correlations support such a function in the testis (Taketo, et al., Devel. Biol. 146:386-395, 1991), where analysis of timing of expression suggests that MIS may be responsible for inhibition of germ cell division. Hutson and Donahoe (Endocrine Reviews 7:270-283, 1986) speculated that MIS may also play role in the transabdominal portion of testicular descent, and Vigier et al. (Development 100:43-55, 1987; Proc. Natl. Acad. Sci. USA 86:3684-8, 1989) have provided evidence that it functions as an inhibitor of aromatase in developing ovaries. Catlin et al. (Am. J. of Obstet. and Gynecol. 159:1299-1303, 1988; Am. Rev. Resp. Dis. 141:466-470, 1990) showed that MIS decreases surfactant accumulation in fetal lungs, thus contributing to the male preponderance in newborn infants of Respiratory Distress Syndrome. The development of a specific serum MIS ELISA (Hudson et al., J. Clin. and Metab. 70:16-22, 1990; Josso et al., J. Clin. Endocrinol. Metab. 70:23-7, 1990) has led to its experimental use as a diagnostic tool for the elucidation of the pathophysiology of ambiguous genitalia in the newborn, and for the use of serum MIS as a marker of granulosa and sex cord tumors in the adult female. Furthermore, the extraordinarily high MIS level observed by Gustafson et al. (New Eng. J. Med. 326:466-71, 1992) in a patient with a sex cord tumor (3200 ng/ml, compared to a normal level of 2-3 ng/ml) provides evidence that MIS is not toxic at these levels.

The role of MIS as a fetal inhibitor has led to the hypothesis that it might act as a tumor inhibitor, particularly of tumors emanating from the Müllerian ducts (Donahoe et al., Science 205:913-915, 1979; Donahoe et al., Ann. Surg. 194:472-480, 1981; Fuller et al., J. Clin. Endocrin. Metab. 54:1051-1055, 1982; Fuller et al., Gynecol. Oncol. 17:124-132, 1984; Fuller et al., Gynecol.

Oncol. 22:135-148, 1985). Experimental evidence has accumulated supporting the ability of recombinant human MIS to exert an antiproliferative effect against genital tract tumors in colony inhibition assays, subrenal capsule assays (Chin, et al., Cancer Research, 51:2101-6, 1991), and now metastases assays, and more recent evidence has shown an antiproliferative effect against a series of human ocular melanomas (Parry et al., Cancer Research 51:1182-6, 1992). MIS has been shown to block tyrosine autophosphorylation of EGF receptors (Coughlin et al., Mol. and Cell. Endocrin. 49:75-86, 1987; Cigarroa et al., Growth Factors 1:179-191, 1989).

Inhibin, another member of the TGF-beta family described above, is primarily secreted by Sertoli and granulosa cells of the male and female gonad. This nonsteroidal regulatory hormone, first described in 1932 (McCullagh, Science 76:19-20), acts specifically to inhibit FSH release from the pituitary (Vale et al., Recent Prog. Horm. Res. 44:1-34, 1988). Biologically active inhibin, however, was not purified and characterized well until the successful cloning of its genes in 1985-86 (Mason et al., Nature 318:659, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091, 1986; Mayo et al., Proc. Natl. Acad. Sci. USA 83:5849, 1986; Esch et al., Mol. Endocrinol. 1:388, 1987). Inhibin was shown at that time to be a glycoprotein heterodimer composed of an alpha-chain and one of two distinct beta-chains (beta-A, beta-B) (Mason et al., Biochem. Biophys. Res. Comun. 135:957, 1986). The alpha chain is processed from an initial species of 57 kDa to form an 18 kDa carboxyl-terminal peptide, while the mature beta chain of 14 kDa is cleaved from the carboxyl-terminus of a 62 kDa precursor, which would then account for the biologically active 32 kDa species which predominates in serum (DeKretser and Robertson, Biol. Reprod. 40:3347, 1989).

Many other forms of bioactive inhibin with MS's of 32-120 kDa, however, have been isolated as well (Miyamoto et al., Biochem. Biophys. Res. Commun. 136:1203-9, 1986). In addition, beta-chain dimers (beta-A/beta-A or beta-A/beta-B) which selectively stimulate FSH secretion from the pituitary have been identified and are called activin A and activin AB, respectively (Vale et al., Nature 321:776, 1986; Ling et al., Nature 321:779, 1986).

As is the case with MIS, many additional functions have been postulated for inhibin and its subunits besides FSH regulation. Inhibin alpha, beta-A, and beta-B subunit RNAs have been shown to be expressed in a variety of rat tissues, including the testis, ovary, placenta, pituitary, adrenal gland, bone marrow, kidney, spinal cord, and brain (Meunier et al., Proc. Natl. Acad. Sci. USA 85:247-51, 1988). The pattern of testicular inhibin secretion appears to be developmentally regulated. In the rat, inhibin increases during maturation until 30-40 days after birth, after which values rapidly return to juvenile levels (Au et al., Biol. Reprod. 35:37, 1986). Inhibin subunits also seem to have a paracrine effect on Leydig and theca interna cell androgen synthesis (Hsueh et al., Proc. Natl. acad. Sci. USA 84:5082-6, 1987). Many studies have demonstrated the changes in inhibin which occur throughout the estrus cycle, and therefore, its role in modulating FSH in adult females (Hasegawa et al., J. Endocrinology 121:91-100, 1989; McLachlan et al., J. Clin. Endo. Metab. 65:954-61, 1987). Furthermore, changes in local inhibin concentrations may be involved in the regulation of ovarian folliculogenesis (Woodruff et al., Science 239:1296-9, 1988; Woodruff et al., Endocrinology 127:3196-205, 1990). Bioactive inhibin has been shown to be produced by human placental cells in culture and to be involved in a short-loop feedback between gonadotropin-releasing hormone and human

chorionic gonadotropin (Petraglia et al., Science 237:187-9, 1987). Finally, a number of patients with ovarian granulosa cell tumors have been described who had markedly elevated serum inhibin levels secondary to tumor production of this hormone (Lappohn et al., NEJM 321:790-3, 1989).

Most of the data that exists concerning serum inhibin levels in humans has been obtained using a heterologous radioimmunoassay comprised of a polyclonal antibody to purified, intact bovine inhibin and radiolabeled 32 kDa bovine inhibin (McLachlan et al., Mol. Cell. Endocrinol. 46:175-85, 1986). Such studies have evaluated normal cycling females and adult males (McLachlan et al., J. Clin. Endo. Metab. 65:954-61, 1987; McLachlan et al., J. Clin. Invest. 82:880-4, 1988), pubertal males (Burger et al., J. Clin. Endo. Metab. 67:689-694, 1988), normal pregnant women (Abe et al., J. Clin. Endocrinol. Metab. 71:133-7, 1990), and a variety of reproductive disorders (Scheckter et al., J. Clin. Endocrinol. Metab. 67:1221-4, 1988; DeKretser et al., J. Endocrinol. 120:517-23, 1989). However, recent work has shown that this assay detects inhibin alpha-subunits as well as intact dimeric hormone, and, therefore, these results should be interpreted with caution (Schneyer et al., J. Clin. Endocrinol. Metab. 70:1208-12, 1990).

Summary of the Invention

The invention features novel isolated DNAs of the TGF-β receptor family, which isolated DNAs encode, for example, MIS receptors, inhibin receptors, and bone morphogenesis protein (BMP) receptors; these receptors are, e.g., those of a mammal such as a rat, mouse, rabbit, guinea pig, hamster, cow, pig, horse, goat, sheep, or human. The invention also includes vectors (e.g., plasmids, phage, or viral nucleic acid) or cells

(prokaryotic or eukaryotic) which contain such DNAs, and the polypeptides produced by expression of such DNAs (for example, by a cell transformed with and capable of expressing a polypeptide from the DNA). By "isolated DNA" is meant a DNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (i.e., one at the 5′ and one at the 3′ end) in the naturally-occurring genome of the organism from which the DNA of the invention is derived. The term thus encompasses, for example, a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment, whether such cDNA or genomic DNA fragment is incorporated into a vector, integrated into the genome of the same or a different species than the organism from which it was originally derived, linked to an additional coding sequence to form a hybrid gene encoding a chimeric polypeptide, or independent of any other DNA sequences. The DNA may be double-stranded or single-stranded, sense or antisense. Examples of isolated DNAs of the invention include those which encode amino acid sequences substantially the same as those shown in Fig. 1 (SEQ ID NO: 14), Fig. 2 (SEQ ID NO: 15), Fig. 3 (SEQ ID NO: 16), and Fig. 4 (SEQ ID NO: 16); and those having sequences which hybridize under conditions of high or moderate stringency to the coding sequence of one of the plasmids included in the ATCC deposit designated No. 75213: misr1, misr2A, misr2B, misr3, or misr4. High stringency conditions are herein defined as the following: hybridizing with 50% deionized formamide, 800 mM NaCl; 20 mM Pipes, pH 6.5, 0.5% SDS, 100 μg/ml denatured, sonicated salmon sperm DNA at 42°C for 12-20 hours, washing with 30 mM NaCl/3.0 mM sodium citrate (0.2 X SSC)/0.1% SDS at 55°C, while moderate stringency conditions are as follows: hybridizing with 50% deionized formamide, 800 mM NaCl; 20 mM Pipes, pH 6.5,

0.5% SDS, 100 μg/ml denatured, sonicated salmon sperm DNA at 42°C for 12-20 hours, washing with 75 mM NaCl/7.5 mM sodium citrate (0.5 X SSC)/0.1% SDS at 55°C.

The isolated DNA of the invention may be under the transcriptional control of a heterologous promoter (i.e., a promoter other than one naturally associated with the given receptor gene of the invention), which promoter, for example, may direct the expression of the DNA of the invention in a particular tissue or at a particular stage of development.

Also within the invention is a substantially pure preparation of an MIS receptor or inhibin receptor protein, or another of the receptor proteins of the invention, prepared, for example, from a natural source, from an expression system expressing the isolated DNA of the invention, or by synthetic means. This protein may, for example, have a sequence the same as, or substantially identical to, that shown in Fig. 1 (SEQ ID NO: 14), Fig. 2 (SEQ ID NO: 15), Fig. 3 (SEQ ID NO: 16), or Fig. 4 (SEQ ID NO: 17), or that encoded by any one of the plasmids deposited as ATCC Accession No. 75213. By "substantially pure preparation" is meant that the preparation is at least 70% free of those proteins with which the protein of the invention is naturally associated in the tissue(s) in which it naturally occurs. In preferred embodiments, the preparation is at least 90% free of such contaminating proteins.

Also within the invention is a substantially pure nucleic acid at least 20 nucleotides in length (preferably at least 50 nucleotides, more preferably at least 100 nucleotides, and most preferably 1000 nucleotides or more in length) which hybridizes under highly stringent conditions to the coding region of a plasmid included in the ATCC deposit designated No. 75213. By "substantially pure nucleic acid" is meant an

RNA or DNA molecule which is substantially free of those other nucleic acid molecules, if any, with which it is naturally associated in the cell from which it was originally derived (i.e., such other nucleic acid molecules make up less than 50% of the total number of nucleic acid molecules in the preparation). By "other nucleic acid molecules" is meant nucleic acid molecules which do not encode the same polypeptide as the nucleic acid of the invention. In preferred embodiments, less than 20%, and more preferably less than 10% of the preparation consists of such other nucleic acid molecules. Such a nucleic acid may be employed in a Northern analysis or *in situ* hybridization assay for determining the level of expression of the gene in a biological sample, which assay would include the steps of (1) providing the isolated DNA of the invention, which isolated DNA includes single stranded antisense DNA; (2) contacting, under hybridizing conditions (preferably of high stringency), the isolated DNA with a biological sample suspected of containing mRNA encoding a receptor of the invention; and (3) determining the level and/or pattern of hybridization of the isolated DNA in the biological sample, the level or pattern of hybridization in the sample being indicative of the level or pattern of expression of the gene encoding the receptor.

As described below, the receptor proteins of the invention (or a ligand-binding portion of such receptors) can be used for a number of purposes. They can be fixed by standard means to a matrix material to form an affinity matrix capable of binding ligand, useful for purifying ligand, for screening for inhibitors of the ligand/receptor interaction, or for determining the amount of ligand present in a given biological sample. They can be used in an assay including the steps of (1) providing the polypeptide of the invention; (2)

contacting the polypeptide with a biological sample suspected of containing MIS, inhibin, or a biologically active fragment thereof; and (3) determining the amount of receptor/ligand complex formation in the sample, such amount of complex formation being indicative of the amount of MIS or inhibin activity in the sample. They can also be used to generate monoclonal or polyclonal antibodies specific for (i.e., capable of forming an immune complex with) such receptors, which antibodies would be useful in a method for detecting the presence of an MIS or inhibin receptor in a biological sample such as serum or tumor cells. Such a method would include the steps of (1) contacting the antibody with a biological sample suspected of containing an MIS or inhibin receptor, and (2) detecting immune complex formation between the antibody and a component of the biological sample, wherein such immune complex formation is indicative of the presence of such a receptor in the sample. Furthermore, such antibodies can be linked to a cytotoxic agent, thereby forming an immunotoxin useful for targeting and killing or disabling cells bearing the receptor of the invention.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Detailed Description

The drawings are first described.

Drawings

Fig. 1 is a representation of the DNA coding sequence of misr1 (SEQ ID NO: 1), and the corresponding amino acid sequence of the encoded receptor protein (SEQ ID NO: 14).

Fig. 2 is a representation of the DNA coding sequence (SEQ ID NO: 2) of two overlapping cloned cDNAs,

misr2A and misr2B, and the corresponding amino acid sequence of the encoded receptor protein (SEQ ID NO: 15).

Fig. 3 is a representation of the DNA coding sequence of misr3 (SEQ ID NO: 3), and the corresponding amino acid sequence of the encoded receptor protein (SEQ ID NO: 16).

Fig. 4 is a representation of the DNA coding sequence of misr4 (SEQ ID NO: 4), and the corresponding amino acid sequence of the encoded receptor protein (SEQ ID NO: 17).

Figs. 5A-E show partial 20 nucleotide sequences of each of misr1 (SEQ ID NO: 5), misr2A (SEQ ID NO: 6), misr2B (SEQ ID NO: 7), misr3 (SEQ ID NO: 8), and misr4 (SEQ ID NO: 9), respectively.

Figs. 6A-F are photographs showing *in situ* hybridization of the urogenital ridge (UGR), ovary, and testis with a riboprobe (R1) derived from misr1 (SEQ ID NO:1) and a second riboprobe (R2) derived from misr2 (SEQ ID NO: 2). Left-hand panels (A,C,E) are representative brightfield views in which hybridization signals appear as black granules (Bar=100μm); right-hand panels (B,D,F) are identical darkfield views in which RNA message appears as bright spots (heavy arrows). A+B) R1 hybridization signal in the 15-day (E15) fetal male UGR is conspicuous over the mesenchyme of the Mullerian duct (M), but not over the adjacent Wolffian duct (W). C+D) R1 signal is also intense over the oocytes (Oo) of preantral and antral follicles (AF) of the postnatal day 20 (P20) ovary, with less intense signal over their adjacent granulosa cells. Two separate R1 riboprobes were used to confirm these finding in Fig. 4A-D: one from the 5′ extracellular domain and one from the 3′ intracellular region of the coding sequence. E+F) R2 signal localizes in a heterogeneous pattern to seminiferous tubules (ST) of the postnatal day 30 (P30)

testis. No R2 message was detected in the fetal Mullerian duct or the pubertal and adult ovary. Both R1 and R2 signals were found in the female postnatal anterior pituitary and hippocampus (data not shown).

Fig. 7 shows the results of Northern analysis of fetal and postnatal rat tissues for MISR1-MISR4 mRNA expression. The left blot was hybridized sequentially with misr1, misr3 and pyruvate kinase (pk) probes, while the right blot was probed serially with misr2a/misr2b, misr4 and pk. Approximately 4.0 kb MISR1, 4.4 and 1.5 kb MISR2, 4.4 kb MISR3, and 6 kb MISR4 transcripts were all detected in the 15-day (E15) fetal urogenital ridge (UGRidge) and postnatal day 1 (P1) testis and ovary. Surprisingly, mRNAs for MISR1, MISR2, and MISR4 were abundant in the 21-day (E21) fetal brain. MISR1-MISR4 message was also present in the E21 fetal lung; other E21 issues, such as the lung, heart, and stomach, contained variable levels of MISR1 and MISR2 mRNA.

Fig. 8 illustrates the results of Northern analysis of a variety of tissues/cells with an misr1 (MIS receptor; SEQ ID NO: 1) cDNA probe. A specific hybridization signal is seen with RNA extracted from rat testicular, ovarian, brain, and pituitary tissues. Lane 1, 21-day fetal rat testes; 2, 21-day fetal rat ovaries; 3, postnatal day 40 rat testis; 4, postnatal day 40 rat ovary; 5, postnatal day 30 male rat pituitary; 6, postnatal day 30 female rat pituitary; 7, postnatal day 1 male rat kidney; 8, postnatal day 1 male rat liver; 9, postnatal day 1 male rat brain; 10, placenta from 15-days gestation; 11, adult ovary from 18 days gestation; 12, human sex cord tumor fragment; 13, A431 human vulvular squamous carcinoma cell line. (10 μg of total RNA per lane, except 2 μg of poly A+ RNA in lane 13; 8 day exposure.)

Fig. 9 illustrates the results of Northern analysis of fetal, prepubertal, pubertal, and adult rat testicular tissue with an misr2 (inhibin receptor; SEQ ID NO: 2) cDNA probe. Maximal hybridization signal was detected with postnatal day 35 and 40 testicular RNA, with a rapid decrease in detectable message by 60 days. This pattern of RNA expression exactly mirrors the known ontogeny of inhibin expression in the maturing rat. Hybridization signal was also detected with rat ovarian and brain tissue (not shown). E15 and E21 samples are from tests collected at days 15 and 21 of gestation, respectively; P7, P14, P20, P24, P27, P30, P35, P40, and P60 samples are all from postnatal animals. (10 μg of total RNA per lane; 4 day exposure.)

Preparation of the isolated DNAs of the invention

Four different isolated DNAs of the invention were prepared by cloning from a rat embryonic urogenital ridge cDNA library, as described below. Some alternative means of preparing the isolated DNAs of the invention, using the information provided herein and standard techniques, are as follows:

(1) A nucleic acid having the nucleotide sequence shown in any one of Figs. 1-4 (SEQ ID NOs: 1-4, respectively), or a nucleic acid encoding the amino acid sequence shown in that figure but, owing to the degeneracy of the genetic code, having a nucleotide sequence different from that shown in the figure, may be synthesized by standard chemical means as generally applied to synthesis of oligonucleotides.

(2) A nucleic acid hybridization probe containing at least 20 nucleotides, and preferably at least 50 nucleotides, of one of the DNA sequences shown in any of Figs. 1-4 (SEQ ID NOs: 1-4) may be prepared by standard methodology and used to probe a "library" of the

five plasmids making up the ATCC deposit designated No. 75213. For example, a probe which includes at least a portion of the nucleotide sequence shown in Fig. 1 (SEQ ID NO: 1), such as the partial sequence shown in Fig. 5A (SEQ ID NO: 5), will hybridize under high stringency conditions (e.g., hybridizing in 50% deionized formamide, 800 mM NaCl, 20 mM Pipes, pH 6.5, 0.4% SDS, 500 μg/ml denatured, sonicated salmon sperm DNA at 42°C for 12-20 hours; and washing in 30 mM NaCl, 3.0 mM sodium citrate, 0.5% SDS at 65°C) solely with a plasmid containing the complementary sequence, and so would identify clones containing the misr1 sequence. Similarly, the partial sequences shown in Figs. 5B, 5C, 5D, and 5E (SEQ ID NOs: 6-9, respectively) can be used to identify misr2A, misr2B, misr3, and misr4, respectively. The desired plasmid can be selected as follows:

The plasmid samples deposited with the ATCC and given accession No. 75123 contain 500ng of each of the five plasmid DNAs in 50 μl final volume. A given clone may be isolated from such a sample by transforming 1μl of DNA from the sample into bacteria HB 101 by either chemical transformation or electroporation. The transformed bacteria are selected on 1.5% agar plates containing 50 μg/ml ampicillin. Ampicillin-resistant colonies are picked individually and grown in 5 ml of LB broth containing 50 μg/ml ampicillin. The plasmid DNA of a few colonies may then be isolated using the standard plasmid DNA mini-prep procedure. The mini-prep DNA is then characterized by means of a DNA dot-blot, using as hybridization probe one of the ^{32}P-labelled misr1, misr2A, misr2B, misr3, or misr4-specific probes discussed above. Alternatively, a cDNA library prepared from a tissue that expresses the gene of interest (such as the rat urogenital ridge cDNA library described below), or a

genomic library from rat, can be probed with such a hybridization probe under highly stringent conditions.

(3) An isolated DNA prepared by any of the methods outlined herein (including the methods originally used to obtain the DNAs of the invention) may be used to probe an appropriate cDNA library or genomic DNA library from any vertebrate species. The stringency of the hybridization conditions would be adjusted as necessary to obtain the desired homolog, while minimizing the number of related but distinct receptor (such as TGF-B or activin receptor) sequences picked up in the assay. It is expected that hybridization and wash conditions such as the highly stringent conditions set forth in (2) above would be adequate; if necessary, the stringency may be increased or decreased, without undue experimentation, using methods well known to those of ordinary skill in the art (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). A given cloned cDNA or genomic DNA would be identified as a homolog of misr1, misr2, misr3, or misr4 by means of sequence comparison, wherein an encoded amino acid sequence that is at least 70% identical to the amino acid sequence encoded by any one of misr1 (SEQ ID NO: 1), misr2 (SEQ ID NO: 2), misr3 (SEQ ID NO: 3), or misr4 (SEQ ID NO: 4) is considered to be a homolog of that receptor. Given the apparently ubiquitous occurrence of MIS, inhibin, and bone morphogenesis proteins (BMPs) among vertebrate species in which they have been sought, it is expected that most or all vertebrate species, and certainly all mammalian species, will be found to have genes encoding at least one MIS receptor, inhibin receptor, and BMP receptor which can be identified by the methods described herein. It is further expected, based upon the information disclosed herein, that many if not all such species will

be found to harbor a plurality of isoforms of such receptor genes.

Each such homolog can be definitively identified as an MIS receptor, inhibin receptor, or BMP receptor by any of the following assays:

(a) Following transient transfection and expression of the putative receptor DNA in an appropriate expression system (i.e., a eukaryotic cell line, such as COS cells, that does not normally express the receptor), the cells are exposed to the suspected ligand (e.g., MIS, inhibin, or one of the BMPs [either recombinant or naturally occurring]) from the same species as the subject homolog receptor. The ligand can be labelled in order to allow detection of binding to the transfected cells (which presumably bear the recombinant receptor on their surfaces), or alternatively a labelled antibody specific for the ligand can be used to indicate whether or not the cells have bound ligand. Binding of the ligand (with or without crosslinking to the receptor) by transfected but not untransfected cells is evidence that the putative receptor DNA does encode a receptor specific for the ligand. Such experiments could be carried out using recombinant human MIS produced as disclosed in Cate et al., U.S. Patent No. 5,047,336 (herein incorporated by reference), and purified by means of an affinity column using an anti-MIS monoclonal antibody, such as disclosed in Donahoe et al., U.S. Patent No. 4,792,601 (herein incorporated by reference). The purified holo MIS is then proteolytically cleaved into an amino terminal fragment and a 24 kDa carboxyl terminal fragment, and the biologically active carboxyl terminal fragment is isolated and radiolabelled. Details of these procedures are provided in the Experimental Data section below. The biologically active form of inhibin (a 32 kDa inhibin carboxyl-terminal fragment) and the various BMPs may also

be radiolabelled as described below. The specific binding and affinity constant can be calculated by using a molar excess of unlabelled ligand for competition.

(b) MIS, inhibin, or any of the BMPs can be fixed to an affinity matrix material by standard methods, and then used to assay for proteins which bind to the matrix: for example, the putative receptor protein expressed by cells transfected with a cloned DNA of the invention, and isolated from the cells' membranes by standard techniques, can be passed over a column of such affinity matrix material. In a variation on this technique, the putative receptor protein itself can be fixed to the matrix material, and a preparation including the ligand (MIS, inhibin, or a BMP) passed over the column.

(c) Eukaryotic cells which do not normally express an MIS, inhibin, or BMP receptor are transfected with the putative receptor DNA of the invention, and used, in accordance with standard procedures, to generate monoclonal antibodies which can differentiate between such transfected cells and identical but untransfected cells. These monoclonal antibodies are then labelled and used in immunohistochemical analysis of given tissues, in order to determine what tissues normally express the putative receptor DNA, and at what stages of development. A pattern of expression that correlates with the expected pattern (the expected pattern being determined, for example, by the pattern of binding of MIS, inhibin, or BMPs in such tissues) would provide evidence that the putative receptor DNA did indeed encode the predicted receptor.

(d) Monoclonal antibodies raised as described above could also be used in a competitive binding assay. A given tissue sample which, by virtue of its ability to bind natural or recombinant MIS, inhibin, or BMP, is known to bear naturally occurring MIS or inhibin

receptors could be employed in a competitive binding assay with either labelled ligand and excess unlabelled antibody raised against the putative receptor (as described above), or labelled antibody and excess unlabelled ligand. Evidence that the ligand and the antibody compete for the same binding sites would support the conclusion that the putative receptor was indeed an MIS, inhibin, or BMP receptor.

(d) Another technique for confirming the identity of a putative receptor of the invention is by the use of Northern blots, probing the RNA of various tissues with a single-stranded hybridization probe made of labelled DNA encoding the putative receptor. The expression of putative receptor-specific genes in tissues known to be affected by MIS, inhibin, BMP, or another candidate ligand, including both normal and disease-state tissues, and the lack of detectible expression in other tissues known to be insensitive to the candidate ligand, is evidence that the putative receptor is indeed a receptor for the candidate ligand.

Use

The cDNAs of the invention, or fragments thereof long enough to serve as specific hybridization probes, can be duplicated by standard means by transfection into appropriate cells (e.g., bacterial cells), purified, and then used as hybridization probes in Northern or in situ hybridization analyses, in order to determine the level of expression of the relevant mRNA in a particular tissue sample. Alternatively, a vector encoding a receptor of the invention plus appropriate expression control elements can be transfected into a cell capable of expressing the receptor polypeptide. Such cells may express the polypeptide as a surface-anchored receptor, or may secrete the polypeptide or accumulate it within

the cell. Purified receptor protein, or cells or membrane preparations bearing the receptor, may be used to generate monoclonal or polyclonal antibodies specific for the given receptor, which antibodies can be employed in assays for detecting the presence or the amount of such receptor in biological samples such as serum or tissue biopsies. Some tumors, including certain ocular melanomas as well as tumors of the female genital tract, are susceptible to the antiproliferative effects of MIS (Donahoe et al., Science 205:913-915, 1979; Donahoe et al., Ann. Surg. 194:472-480, 1981; Fuller et al., J. Clin. Endo. Metab. 54:1051-1055, 1982; Fuller et al., Gynecol. Oncol. 22:135-148, 1985; Chin et al., Cancer Res. 51:2101-2106, 1991; Parry et al., Cancer Res. 52:1182-1186, 1992; and Donahoe, U.S.S.N 683,966, herein incorporated by reference), and it is postulated that the growth of other tumor types may be similarly reduced by inhibin or BMP. The antibodies of the invention would therefore be useful for identifying candidate tumors likely to respond to therapy with MIS, inhibin, BMP, or agonists or antagonists thereof. The receptor polypeptides of the invention, and their respective antibodies, could be used as receptor agonists or antagonists in the management of relevant clinical disorders. The antibodies can also be used as the targeting means for directing cytotoxic agents to cells (such as tumor cells) bearing the given receptor. Examples of cytotoxic agents commonly used in such applications include, for example, polypeptide toxins such as diphtheria toxin, *Pseudomonas* exotoxin A, ricin, and gelonin, or defined toxic portions thereof; radioisotopes; and agents such as cisplatinum, adriamycin, bleomycin, and other therapeutic cytotoxins. Methods for making such immunotoxins are well known to those of ordinary skill in the art, and may include

genetic engineering technology as well as chemical-based techniques.

Purified receptor protein, or transformed cells expressing the receptor protein, can be used to screen candidate drugs for their ability to block or enhance the binding of MIS, inhibin, or BMPs to their respective receptors. This could be accomplished by means of a competition assay using, for example, labelled ligand and excess candidate drug. Inhibitors of MIS ligand/receptor binding would potentially be useful for preventing or alleviating respiratory distress syndrome in newborns (Donahoe et al., U.S.S.N. 416,235, herein incorporated by reference). Substances which act as inhibitors of inhibin/receptor binding could be used for treatment of infertility: for example, the extracellular domain of a soluble inhibin receptor can act as an inhibin antagonist, thereby increasing the level of FSH in infertile patients with low FSH. Inhibitors of BMP/receptor binding (such as the extracellular domain of a BMP receptor) could be used in a similar fashion to enhance the action of bone-specific trophic factors.

Recombinant forms of the MIS receptor, inhibin receptor, or BMP receptors, or ligand-binding portions thereof, can be used to measure the amount of ligand (MIS, inhibin, or one of the BMPs) present in a biological sample. This could be accomplished, for example, by means of a sandwich assay utilizing the recombinant receptor protein fixed to a solid support, and labelled anti-ligand antibody. Where the ligand being measured is MIS, it may be desireable to include plasmin or an MIS-specific protease in the assay, in order to permit the cleavage of any holo MIS present in the sample into its receptor-binding form. The recombinant receptors of the invention would also be useful as a means for assaying receptor binding by

analogs of MIS, inihibin, and the BMPs, in order to develop analogs with an enhanced affinity for the given receptor. Those analogs which are capable of stimulating a signal through the receptor can then be used in MIS, inhibin or BMP replacement therapy, while those analogs which bind but do not activate the given receptor will be useful as inhibitors of the natural ligand.

The receptors of the invention may also have therapeutic applications. Where a given condition, such as respiratory distress syndrome in newborns, is attributable to an overabundance of MIS in a given tissue, exposure of that tissue to recombinant MIS receptor protein, or a soluble, MIS-binding fragment thereof, provides a means for reducing the amount of MIS available for binding to natural receptors in the tissue and thereby alleviating the underlying cause of the condition. Similarly, a soluble, inhibin-binding fragment of the inhibin receptor would be useful, as discussed above, for increasing the level of FSH in patients with infertility attributable to abnormally low FSH levels. A soluble, BMP-binding fragment of a BMP receptor could be utilized in an assay to measure the amount of a particular BMP present in a biological sample: for example, to determine whether BMP supplemental therapy would be called for in a given case of retarded bone growth or repair of traumatic bone injuries or deficiency due to removal of bone in surgery for a malignancy or other deformities. Such soluble receptor fragments can be readily produced by genetically engineering the receptor cDNAs of the invention to delete those portions encoding the largely hydrophobic putative transmembrane regions, but leaving intact the sequences encoding the putative extracellular domains. Such methods are well known in the art. One example of a soluble fragment of MISR1 would include most or all of

amino acids 1 to 510 of the sequence shown in Fig. 1 (SEQ ID NO: 14), but would not include amino acids 121 to 138. Alternatively, a given soluble receptor fragment may be produced by proteolytic treatment of naturally occurring or recombinant membrane-bound MIS or inhibin receptors. Such soluble fragments can be assayed for their ability to bind to ligand by the use of radiolabelled ligand or ligand fixed to affinity matrix.

Deposit

Under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure, a deposit of plasmids misr1, misr2A, misr2B, misr3, and misr4 has been made with the American Type Culture Collection (ATCC) of Rockville, MD, USA, where the deposit was given Accession No. 75213.

Applicants' assignee, the General Hospital Corporation, represents that the ATCC is a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. The material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. §122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited material, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent, whichever period is longer. Applicants' assignee acknowledges its duty to replace the deposit should the depository be unable to

furnish a sample when requested due to the condition of the deposit.

Experimental Data

Four novel membrane serine/threonine kinase receptor cDNAs from the rat urogenital ridge were cloned and characterized as described below.

Polymerase chain reaction (PCR) using consensus primers.

The DNA sequence of the cDNA encoding a murine activin receptor (Mathews and Vale, Cell 65:973-982, 1991) was compared to that of certain related cDNAs: human and porcine TGF-β type II receptor (Lin et al., Cell 68:775-785, 1992) and the daf-1 receptor of C. elegans (Georgi et al., Cell 61:635-645, 1990), and two highly conserved regions defined. These two regions formed the basis for the design of two degenerate oligonucleotides:

5'- GTGGCCGT(G/C)AA(A/G)AT(C/T)TT - 3' (SEQ ID NO: 10)

and 5'- GAC(T/C)TCTGG(G/A)GCCAT(G/A)TA - 3' (SEQ ID NO: 11).

The oligonucleotides were synthesized with an Applied Biosystems 391 DNA synthesizer, and used as primers for polymerase chain reaction (PCR)-based selection from a 14.5 day rat urogenital ridge COS cell expression cDNA library. PCR was carried out in a 50 μl reaction mixture containing about 1 μg of cDNA plasmid; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 5 mM $MgCl_2$; 0.001% gelatin; 250 μM each of dATP, dCTP, dGTP, and dTTP; 1 unit of Taq polymerase (Perkin-Elmer Cetus); and 50 pmol each of the above oligonucleotides. Thirty cycles of PCR (consisting of denaturation at 94°C for 1 min; annealing at 37°C for 1 min; and elongation at 72°C for 1 min) were performed. The PCR products were separated on a 1.5% agarose gel and a predicted 400-500 bp DNA fragment was sliced out and

purified by Gene-clean™. The purified PCR product was blunt-ended with Klenow fragment and phosphorylated with T4 polynucleotide kinase. The final PCR fragment was ligated, using T4 DNA ligase, with plasmid pGEM7Z(+) vector which was digested with Sma I and dephosphorylated. The ligation mixture was incubated at room temperature for 3 hours, and then transformed into bacteria HB 101 by electroporation. Bacterial colonies resistant to ampicillin were selected overnight on 1.5% agar plates containing 50 μg/ml ampicillin. Individual colonies were picked and grown in 5 ml of LB broth, and plasmids were isolated according to a standard plasmid mini-prep protocol. The plasmid DNA was then sequenced with bacterial phage promoter SP6 and T7 primers using Sequenase (USB). Four clones containing PCR fragments encoding portions of four novel polypeptides (putative serine/threonine kinases) were designated pGEM7-Misr1, pGEM7-Misr2, pGEM7-Misr3 and pGEM7-Misr4, respectively. In addition, cDNAs encoding portions of TGF-beta receptor and activin receptor were isolated during this procedure; these were designated pGEM7-tgfb and pGEM7-actr, respectively.

cDNA Library Synthesis

Approximately 450 urogenital ridges and their adjacent gonads were collected from 24 litters of 14.5-15 day gestational age fetal rats, and flash frozen in liquid nitrogen. RNA was then extracted from this tissue by homogenization in 50% guanidinium thiocyanate/14.5% lithium chloride/0.2% β-mercaptoethanol, centrifugation through 5.7 M cesium chloride (50k rpm for 2 hours), and precipitation with NaOAc and ethanol. Poly A+ RNA was further obtained by oligo dT - cellulose chromatography of 620 μg total RNA. Twenty μg of this poly A+ RNA was subsequently used for first strand cDNA synthesis, using 4 μl of reverse

transcriptase (RT-XL, Life Sciences), 2.5 μl of 20 mM ultrapure dNTP, 1 μl of oligo dT (Collaborative Research, 5 μg/μl) as primer, 20 μL of RT1 buffer, 1 μL 1.0 M DTT, and 2 μl of placental RNase inhibitor (Boehringer, 36 U/μl) in a total volume of 100 μl, incubated for forty-five minutes at 42°C. The second strand synthesis reaction, which employed 5 μl of DNA polymerase I (Boehringer, 5 U/μl) and 2 μl of RNase H (BRL, 2 U/μl), was performed for one hour at 15°C followed by one hour at 22°C, prior to termination with 20 μl of 0.5 M EDTA, pH 8.

The cDNA mixture was then phenol extracted and ethanol precipitated, and then ligated to non-self-complimentary BstX1 linkers (Invitrogen) using 1 μl of T4 DNA ligase (NE Biolabs, 400 U/μl), in a volume of 50 μl incubated at 15°C overnight. Small cDNA and free linkers were removed by centrifugation through a 5-20% KOAc gradient. Fractions of the gradient that contained cDNA larger than one kilobase were ethanol precipitated with linear polyacrylamide and pooled. After test ligations had determined the optimal ratios, the cDNA was ligated into the COS cell expression vector CDM8, previously digested with BstX1. The cDNA/vector products were electroporated (BioRad Gene Pulser) into competent E. coli MC1061/p3 cells, which were then grown on 20 LB/ampicillin bacterial plates. The resulting cDNA library contained 1 X 10^6 individual clones, with an average insert size of 1.5-2.0 kb. Maxiprep plasmid DNA (total yield 1.9 mg) was subsequently obtained from a "pooled" overnight liquid culture of these clones.

A lambda Zap II library was constructed by Stratagene using 20 μg of urogenital ridge mRNA.

Cloning of the full-length cDNAs for Misr1, Misr3 and Misr4, and two partial cDNAs for Misr2

The plasmid DNA of each of pGEM7-misr1, pGEM7-misr2, pGEM7-misr3, and pGEM7-misr4 was prepared in large quantities according to a standard plasmid large-prep protocol. The inserts of individual clones were excised out of the plasmid vector with restriction enzymes Eco RI and Bam HI. The inserts were then gel-separated and purified with Gene-clean™. The purified DNA inserts were labeled with ^{32}P-dCTP using a random-priming technique, to a specific activity of greater than 1 x 10^9 cpm/μg. The individually labeled DNA probes were then used to screen a 14.5 day rat urogenital ridge lambda ZAP II cDNA library made by Stratagene. Positive clones were plaque-purified and the inserts were excised into plasmid pBluescript I SK according to Stratagene's protocol. Full-length clones were sequenced with Sequenase on both strands by synthesizing internal 16-17 oligonucleotide sequencing primers. The full-length DNA coding sequence of misr1 (SEQ ID NO: 1) and the amino acid sequence of its encoded polypeptide (referred to as MISR1; SEQ ID NO: 14) are shown in Fig. 1. The full coding sequence of misr2A/misr2B is shown in Fig. 2 (SEQ ID NO: 2), where the overlap between the two cloned sequences is indicated. The full length polypeptide encoded by a DNA sequence resulting from the ligation of appropriate portions of misr2A and misr2B to produce a single, full-length coding sequence is also shown in Fig. 2; this full-length polypeptide is referred to herein as MISR2 (SEQ ID NO: 15). Full-length sequences of clones misr3 and misr4 are shown in Fig. 3 (SEQ ID NO: 3) and Fig. 4 (SEQ ID NO: 4), respectively. The full-length polypeptide encoded by misr3 is termed MISR3 (shown in SEQ ID NO: 16), while the full-length polypeptide encoded by misr4 is termed MISR4 (shown in SEQ ID NO: 17). Each

sequence was compared to sequences in the GenBank database, and found to be unique. Misr1 (SEQ ID NO: 1) is believed to encode an isoform of the rat MIS receptor, while misr2A/misr2B (SEQ ID NO: 2), misr3 (SEQ ID NO: 3), and misr4 (SEQ ID NO: 4) are believed to encode monomeric isoforms of the rat inhibin receptor and/or BMP receptor.

Each putative receptor of 501-509 amino acid residues possesses the characteristic domain features of the TGF-β receptor superfamily, including a hydrophobic signal peptide of 19-23 residues (von Heijne, Biochim. Biophys. Acta 947:307, 1988); an extracellular, cysteine-rich, hydrophilic, ligand-binding domain of 100-150 residues, a hydrophobic single transmembrane domain of 23-25 residues (Kyte et al, J. Mol. Biol. 157:105, 1982), an intracellular serine/threonine kinase domain of approximately 300 residues, and a short serine/threonine rich tail. Sequence alignment with the TGF-β and activin type II receptors and daf-1 reveals greatest the similarity between their intracellular domains, including conservation of 22 amino acid residues that are characteristic of the serine/threonine subfamily of protein kinases (Hanks, Meth. Enzymol. 200:38, 1991). All such kinases, including members of the TGF-β receptor family and MISR1-MISR4, have 12 subdomains of highly conserved residues. For example, $GXGXXGXVX_{11-28}K$, conserved in subdomains I and II and thought to form an ATP binding site, aligns well in MISR1-MISR4 as $GKGR(Y/F)GEVX_{12}K$ (SEQ ID NOs: 12 and 13). Subdomains VIB and VIII are key regions which determine tyrosine and serine/threonine kinase specificity; in each of MISR1-MISR4, these domains are more homologous to the serine/threonine motif than to the tyrosine sequence (Hanks et al., Science 241:42-52, 1988).

In situ Hybridization

Plasmids pGEM7-Misr1, pGEM7-Misr2, pGEM7-Misr3, and pGEM7-Misr4 were linearized with appropriate restriction enzymes. Antisense or sense RNA probes labelled with [^{35}S]-UTP were generated by transcription of the linearized plasmid DNA using the Riboprobe Gemini System II (Promega Biotech) with SP6 or T7 RNA polymerases.

Tissue sections were postfixed in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4, for 5 minutes at room temperature, then rinsed twice in PBS. The sections were rinsed briefly with 0.1M triethanolamine-HCl, pH 8.0, and then treated with 0.25% acetic anhydride in 0.1M triethanolamine-HCl, pH 8.0, for 10 min. at room temperature. The sections were rinsed twice in 2X sodium chloride/sodium citrate (SCC), then dehydrated in increasing concentrations of ethanol, delipidated in chloroform, rehydrated, and air dried for 30 min. at room temperature. Sections were hybridized under coverslips for 15 hours at 55°C using ^{35}S-labelled sense or antisense probe (2 x 10^7 cpm/ml) in 50% formamide, 600 mM NaCl, 10 mM Tris-HCl (pH 7.5), 0.02% Ficoll, 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, 1 mM EDTA, 0.01% salmon testis DNA, 0.05% total yeast RNA, 0.005% yeast tRNA, 10% dextran sulfate, 0.1% SDS, 0.1% sodium thiosulfate, and 100 mM DTT. After hybridization, slides were immersed in 2X SSC for 30 min. at room temperature, and floated off the coverslips. The slides were first treated with RNase A (20 mg/ml) in RNase buffer (0.5 M NaCl, 10 mM Tris-HCl, pH 8.0, 1.0 mM EDTA) for 30 min. at 37°C and washed in the same buffer for 30 min. at 37°C. The slides were then washed in 2X SSC for 1 hour at 50°C, 0.2X SSC for 1 hour at 55°C, 0.2X SSC for 1 hour at 60°C, then dehydrated sequentially in 70%, 80%, and 95% ethanol

containing 300 mM ammonium acetate, and absolute ethanol before air drying. To detect autoradiographic silver grains, the slides were dipped into Kodak NTB-2 nuclear track emulsion diluted 1:1 with 0.1% Aerosol 22 (Sigma) at 42°C, dried gradually in a high humidity chamber for 2 hours, then exposed at 4°C for 7-14 days. The slides were developed in Kodak D19 for 2 min. at 16°C, rinsed in deionized water for 30 sec., fixed in Kodak fixer for 5 min., then washed in deionized water and stained with hematoxylin. Sections were examined using bright and darkfield illumination.

To identify potential ligands for MISR1-MISR4 binding studies, *in situ* hybridization was performed with 13 to 16-day fetal urogenital ridge and fetal, peripubertal, and adult gonads (Fig. 6). Remarkably, misr1 was the only clone to localize specifically to 14.5 to 15-day fetal male Mullerian duct mesenchyme, but not to the adjacent Wolffian duct or gonad or to 13 or 16-day Mullerian tissue. This was a consistent finding using misr1 riboprobes derived from either the 3′ conserved domain or the 5′ extracellular region, making cross-hybridization with homologous receptors unlikely. In addition, misr1 message localized to oocytes of preantral and antral follicles of the peripubertal and adult ovary. Because the expression and ontogeny of misr1 mRNA is consistent with both the known site (Trelstad et al., Develop. Biol. 92:27-40, 1982; Tsuji et al., Endocrinology 131:1481-1488, 1992) and timing (Picon, Arch. Anat. Micro. Morphol. Exp. 58:1-19, 1969) of MIS action in the urogenital ridge, as well as the cycling adult ovary (Takahashi et al., Molec. Cell. Endocr. 47:225-234, 1986; Ueno et al., Endocrinology 125:1060-1066, 1989), MISR1 is the best candidate for the rat MIS receptor. MISR2 mRNA, on the other hand, localized in a heterogeneous pattern to seminiferous tubules of pubertal

and adult testes, but was not detectable within the fetal or adult ovary (Figs. 6E and 6F). Both MISR1 and MISR2 transcripts were also observed in the postnatal female anterior pituitary and hippocampus (data not shown), but their cellular localization has not been clearly delineated.

Northern Analysis

Northern analysis of a variety of fetal and adult rat tissues was performed to determine both the tissue and temporal specificity of expression of RNA corresponding to each of the four newly identified receptor clones. Total RNA was extracted by a modification of the method of Chirgwin using guanidinium thiocyanate/lithium chloride; RNA quantification was by spectrophotometric analysis and ethidium bromide staining of test gels. Ten μg of total RNA (or in selected cases, 1 μg of poly-A+ RNA) were loaded in each lane of 1.5% Morpholinopropanesulfonic acid-formaldehyde agarose gels, electrophoresed at 5 V/cm, transferred to Biotrans nylon membranes (ICN Biomedicals, Irvine, CA) by capillary action in 25 mM sodium phosphate, and then fixed by UV irradiation.

Membranes were prehybridized in plaque screen buffer (0.05 M Tris-Cl, 0.1% Na pyrophosphate, 1 M NaCl, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 0.2% BSA, 1% SDS) containing 0.1 mg/ml tRNA for 2 hours at 65°C. Membranes were then hybridized with one of the four randomly primed, ^{32}P-labeled receptor cDNA clones, which varied in length from 0.5 to 3.0 kb. Overnight hybridization was performed with $1x10^6$ cpm/ml in plaque screening buffer containing 0.1 mg/ml tRNA. All hybridizations and washes were done at 65°C; 30 mM NaCl/3.0 mM Na citrate/0.5% SDS was the most stringent wash. Autoradiographic exposures were for 3-10 days.

As shown in Fig. 7, mRNA transcripts of 4.0 kb (misr1), 4.4 and 1.5 kb (misr2A/misr2B), 4.4 kb (misr3), and 6 kb (misr4) were detected in 15-day (E15) fetal urogenital ridge tissue and postnatal day 1 (P1) testis and ovary. Similar levels of expression were found for each clone in pubertal and adult gonads. misr1, misr2, and misr4 message was also abundant in the 21-day (E21) fetal brain, with misr1 mRNA persisting in the adult female brain (data not shown). Interestingly, all four of these mRNAs are present in the E21 lung (particularly misr3 and misr4) and persist there to adulthood (data not shown). Transcripts for misr1 and misr2, and less so for misr3, were detected in other E21 tissues such as the lung, heart, and stomach, suggesting a more universal distribution of these receptors than anticipated.

As illustrated in Figs. 8 and 9, the misr1 (MIS receptor) probe hybridized to mRNA from testes, ovary, brain, and pituitary, while the misr2 (inhibin receptor) probe hybridized with testicular RNA in a distinctive temporal pattern. Misr2 probe was also found to hybridize to ovarian and brain tissue (data not shown). These results are consistent with the conclusion that misr1 encodes the rat MIS receptor, while misr2A/2B together encode the rat inhibin receptor.

Holo RhMIS Purification

Recombinant human MIS (rhMIS) purification by immunoaffinity chromatography from conditioned media of Chinese hamster ovary cells transfected with human MIS gene is as follows. Media were collected every 3-4 days from bioreactor cultures (Epstein et al., In Vitro Cell. Dev. Biol. 25:213-216, 1989), and stored at -20°C until use. A 5 ml immunoaffinity column was constructed using approximately 50 mg of the protein A-Sepharose (Sigma Chemical Co., St. Louis, MO) purified mouse monoclonal anti-human rhMIS antibody (Hudson et al., J. Clin.

Endocrinol. Metab. 70:16-22, 1990) covalently attached to Affigel-10 agarose resin (BioRad Laboratories, Richmond CA). The column was equilibrated with 100 ml of 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 7.4, and 200 ml of concentrated medium loaded after filtration through Whatman #4 paper at 1 column volume/h at 4°C. After loading, the column was washed with 20 mM Hepes, pH 7.4, until the absorbance at 280 nm returned to baseline (60-100 ml).

RhMIS was eluted using 1 M acetic acid in 20 mM Hepes, pH 3.0, after a one column volume pre-elution wash containing 0.5 M NaCl, 1 mM EDTA, 0.001% nonidet P-40 (NP-40, Sigma Chemical Co., St. Louis, MO), 20 mM Hepes, pH 7.4. The majority of the rhMIS eluted in a single 2 ml fraction, which was immediately neutralized with NaOH to a pH between 7.0 and 7.4. The acid-eluted immunoaffinity-purified (IAP) fractions were dialyzed overnight versus 0.02 M Hepes, 0.001% NP-40, pH 7.4. The resulting samples were analyzed for total protein by the Bradford method (Bradford, Anal. Biochem. 72:248-254, 1976) and for rhMIS concentrations by an enzyme-linked immunosorbent assay (Hudson et al., J. Clin. Endocrinol. Metab. 70:16-22, 1990). They were further examined by polyacrylamide gel electrophoresis (Weber et al., J. Biol. Chem. 244:4406-4412, 1969) and activity determined in an *in vitro* Müllerian duct regression bioassay.

Purification of the carboxyl-terminus of rhMIS

Immunoaffinity purified rhMIS (1.1-1.5 mg in 2.5 ml of 20 mM Hepes buffer, pH 7.4) was incubated with plasmin (EC 3.4.21.7, Sigma Chemical Co., St. Louis, MO) at a ratio of 20 to 25:1 rhMIS to plasmin w:w for 2 hr at room temperature as previously described (Pepinsky et al., J. Biol. Chem. 263:18961-18964, 1988). The preparation was then placed onto a 2.5 x 16 cm P-100 polyacrylamide column (BioRad Laboratories, Richmond, CA)

equilibrated at 4°C with 1.0 M acetic acid in 20 mM Hepes at pH 3.0. Protein was eluted in 0.54 ml fractions at a flow rate of approximately 2.0 ml/hr. Ten microliter aliquots were analyzed for protein by the Bradford method (Bradford, Anal. Biochem. 72:248-254, 1976). Two peaks of protein, termed A and B, elute from this column. These peaks were pooled separately, frozen in liquid nitrogen, and concentrated by lyophilization in a Savant Speed Vac apparatus. The resulting pools were dissolved in either 20 mM Hepes, pH 7.4, or 0.3 M sodium phosphate, pH 7.4, so that a final protein concentration of 1 mg/ml was achieved. Elution buffer in volumes similar to those of the pools was also lyophilized and dissolved in buffer as above to serve as controls for the rhMIS bioassays.

Rh MIS Bioassay

The standard organ culture bioassay for MIS was performed as described (Donahoe et al., Biol. Reprod. 16:238-243; MacLaughlin et al., Methods in Enzymology 198:358-369, 1991). Briefly, 14½ day female fetal rat urogenital ridges were placed on agar-coated stainless steel grids above fortified CMRL 1066 medium (GIBCO/BRL, Gaithersburg, MD) containing female fetal (and therefore MIS-free) calf serum (Necklaws et al., Endocrinology 118:791-796, 1986) and testosterone at 10^{-9} M, to enhance the Wolffian duct for direct comparison of the Müllerian duct in each tissue section. RhMIS protein samples of 0.5 to 8.0 μg each, or buffer controls, were added in serum containing CMRL medium after sterile filtration in that solution through a 0.22 μm Millex GV membrane. Control studies using carboxyl-terminal rhMIS radiolabeled with I^{125} by a standard technique (Hunter, Proc. Soc. Exp. Biol. Med. 133:989-992, 1970) demonstrated no loss of the protein to this filter. After incubation for 3 days in humidified 5% CO_2 at 37°C, the specimens were fixed in 15% formalin, embedded in

paraffin, and 8 μm sections of the cephalic end stained with hematoxylin and eosin. The sections were then ranked from grade 0 (no regression) to grade 5 (complete regression), by two experienced observers. One unit of activity is defined as causing a 1 grade increase in Müllerian duct regression. Data were compared by Student's t-test for significant differences among groups.

Radioisotope labelling of ligand

Iodination of both MIS and inhibin carboxyl terminal fragments is performed with ^{125}I Na and chloramine-T. One to five μg of protein is suspended in 0.3 M sodium phosphate buffer, pH 7.5, and radioisotope then added at a ratio of 1mCi:5μg. Three serial additions of chloramine-T solution are next performed, with a final chloramine-T to protein ratio of 1:7 and a total reaction time of 4.5 minutes. The reaction is terminated with saturated potassium iodide solution containing 0.1% BSA; free isotope is then separated from radiolabeled ligand by size exclusion chromatography. Estimated specific activities of 50-70 x 10^6 cpm/μg have been obtained for both ligands using this method.

Other embodiments are within the following claims.

What is claimed is:

1. Isolated DNA comprising a sequence encoding a Müllerian Inhibitory Substance (MIS) receptor.

2. The isolated DNA of claim 1, wherein said receptor is a mammalian protein.

3. The isolated DNA of claim 1, wherein said receptor is a human protein.

4. The isolated DNA of claim 1, wherein said receptor is a rat protein.

5. The isolated DNA of claim 4, wherein said receptor has substantially the amino acid sequence of MISR1 (SEQ ID NO: 14).

6. The isolated DNA of claim 1, wherein said sequence encoding said receptor hybridizes under high stringency conditions with the coding sequence of a plasmid contained in the ATCC deposit designated No. 75123.

7. An isolated DNA comprising a 20-nucleotide sequence which hybridizes under high stringency conditions with the coding sequence of misr1 (SEQ ID NO: 1).

8. A cell comprising the isolated DNA of claim 1.

9. The cell of claim 8, wherein said cell is capable of expressing said receptor.

10. The cell of claim 8, wherein said cell is a eukaryotic cell.

11. The isolated DNA of claim 1, wherein said sequence encoding said receptor is under the transcriptional control of a heterologous promoter.

12. A substantially pure nucleic acid at least 20 nucleotides in length which hybridizes under high stringency conditions to the coding region of a plasmid included in the ATCC deposit designated No. 75213.

13. A vector comprising a nucleotide sequence at least 20 nucleotides in length which hybridizes under high stringency conditions to the coding region of a plasmid included in the ATCC deposit designated No. 75213.

14. The vector of claim 13, wherein said vector is a viral nucleic acid.

15. A substantially pure preparation of an MIS receptor protein.

16. A substantially pure preparation of a polypeptide having an amino acid sequence substantially identical to that shown in Fig. 1 (SEQ ID NO: 14).

17. A substantially pure polypeptide encoded by the isolated DNA of claim 7, wherein said isolated DNA comprises a 100-nucleotide sequence which hybridizes under high stringency conditions with the coding sequence of misr1 (SEQ ID NO: 1).

18. The polypeptide of claim 17, wherein said isolated DNA comprises a 1000-nucleotide sequence which hybridizes under high stringency conditions with the coding sequence of misr1 (SEQ ID NO: 1).

19. An affinity matrix comprising a polypeptide encoded by the isolated DNA of claim 7, wherein said isolated DNA comprises a 100-nucleotide sequence which hybridizes under high stringency conditions with the coding sequence of misr1 (SEQ ID NO: 1).

20. An antibody which forms an immune complex with an MIS receptor.

21. A method of detecting the presence of an MIS receptor in a biological sample, said method comprising:

contacting the antibody of claim 20 with a biological sample suspected of containing an MIS receptor, and

detecting immune complex formation between said antibody and a component of said biological sample, wherein said immune complex formation is indicative of the presence of an MIS receptor in said biological sample.

22. The method of claim 21, wherein said biological sample comprises tumor cells.

23. The method of claim 21, wherein said biological sample is serum.

24. A method of determining the level of expression of a gene in a biological sample, said method comprising:

providing the isolated DNA of claim 1, said isolated DNA comprising single stranded antisense DNA;

contacting, under hybridizing conditions, said isolated DNA with a biological sample suspected of containing mRNA encoding an MIS receptor; and

determining the level of hybridization of said isolated DNA with said biological sample, said level of hybridization being indicative of the level of expression in said biological sample of a gene encoding said MIS receptor.

25. A method for determining the amount of MIS activity in a biological sample, said method comprising:

providing a substantially pure preparation of an MIS receptor or MIS-binding fragment thereof;

contacting said receptor or fragment with a biological sample suspected of comprising MIS or a biologically active fragment of MIS; and

determining the amount of receptor/ligand complex formation in said biological sample, said amount of complex formation being indicative of the amount of MIS activity in said biological sample.

26. An immunotoxin comprising the antibody of claim 20 linked to a cytotoxic agent.

27. The immunotoxin of claim 26, wherein said antibody is chemically conjugated to said cytotoxic agent.

28. The immunotoxin of claim 26, wherein said cytotoxic agent is a polypeptide toxin.

29. Isolated DNA comprising a sequence encoding an inhibin receptor.

30. The isolated DNA of claim 29, wherein said receptor is a mammalian protein.

31. The isolated DNA of claim 30, wherein said receptor is a human protein.

32. The isolated DNA of claim 30, wherein said receptor is a rat protein.

33. The isolated DNA of claim 30, wherein said receptor is MISR2.

34. A cell comprising the isolated DNA of claim 29.

35. The isolated DNA of claim 29, wherein said sequence hybridizes under high stringency conditions with a sense or antisense strand of DNA encoding the amino acid sequence given in Fig. 2 (SEQ ID NO: 15).

36. The isolated DNA of claim 29, wherein said sequence hybridizes under high stringency conditions with the coding sequence of a plasmid contained in the ATCC deposit designated No. 75213.

37. The isolated DNA of claim 29, wherein said sequence encoding said receptor is under the transcriptional control of a heterologous promoter.

38. A substantially pure preparation of an inhibin receptor.

39. A substantially pure polypeptide having an amino acid sequence substantially identical to that shown in Fig. 2 (SEQ ID NO: 15).

40. A substantially pure polypeptide encoded by the isolated DNA of claim 29, wherein said isolated DNA

comprises a 100-nucleotide sequence which hybridizes under high stringency conditions with the coding sequence of misr2 (SEQ ID NO: 2).

41. The polypeptide of claim 40, wherein said isolated DNA comprises a 1000-nucleotide sequence which hybridizes under high stringency conditions with the coding sequence of misr2 (SEQ ID NO: 2).

42. An affinity matrix comprising the polypeptide of claim 40.

43. An antibody which forms an immune complex with an inhibin receptor.

44. A method of detecting the presence of an inhibin receptor in a biological sample, said method comprising:

contacting the antibody of claim 43 with a biological sample suspected of containing an inhibin receptor, and

detecting immune complex formation between said antibody and a component of said biological sample, wherein said immune complex formation is indicative of the presence of an inhibin receptor in said biological sample.

45. A method for determining the amount of inhibin activity in a biological sample, said method comprising:

providing a substantially pure inhibin receptor protein or inhibin-binding fragment thereof;

contacting said receptor protein or fragment with a biological sample suspected of comprising inhibin or a biologically active fragment of inhibin; and

determining the amount of receptor/ligand complex formation in said biological sample, said amount of complex formation being indicative of the amount of inhibin activity in said biological sample.

46. An immunotoxin comprising the antibody of claim 43 linked to a cytotoxic agent.

47. Isolated DNA comprising a sequence which hybridizes under high stringency conditions with a sequence encoding MISR3 (SEQ ID NO: 16).

48. The isolated DNA of claim 47, wherein said sequence encoding MISR3 is misr3 (SEQ ID NO: 3).

49. The isolated DNA of claim 47, wherein said sequence which hybridizes under high stringency conditions encodes a human receptor protein.

50. The isolated DNA of claim 49, wherein said human receptor protein is a bone morphogenic protein (BMP) receptor.

51. A substantially pure polypeptide encoded by the isolated DNA of claim 47.

52. The polypeptide of claim 51, wherein said polypeptide is a BMP receptor.

53. Isolated DNA comprising a sequence which hybridizes under high stringency conditions with a sequence encoding MISR4 (SEQ ID NO: 17).

54. The isolated DNA of claim 53, wherein said sequence encoding MISR4 is misr4 (SEQ ID NO: 4).

55. The isolated DNA of claim 53, wherein said sequence which hybridizes under high stringency conditions encodes a human receptor protein.

56. The isolated DNA of claim 55, wherein said human receptor protein is a bone morphogenic protein (BMP) receptor.

57. A substantially pure polypeptide encoded by the isolated DNA of claim 53.

58. The polypeptide of claim 57, wherein said polypeptide is a bone morphogenic protein (BMP) receptor.

FIG. 1

```
                  560                 580                 600
atggtcgatggagcaatgatcctttctgtgctaatgatgatggctctcccttccccgagt
MetValAspGlyAlaMetIleLeuSerValLeuMetMetMetAlaLeuProSerProSer
                  620                 640                 660
atggaagatgaggagcccaaggtcaacccgaagctttacatgtgtgtgtgtgagggcctc
MetGluAspGluGluProLysValAsnProLysLeuTyrMetCysValCysGluGlyLeu
                  680                 700                 720
tcctgcgggaacgaggaccactgtgagggccagcagtgtttttcctccctgagcgtcaat
SerCysGlyAsnGluAspHisCysGluGlyGlnGlnCysPheSerSerLeuSerValAsn
                  740                 760                 780
gatggcttccgcgtctaccagaagggctgctttcaggtctatgagcaggggaagatgacg
AspGlyPheArgValTyrGlnLysGlyCysPheGlnValTyrGluGlnGlyLysMetThr
                  800                 820                 840
tgtaagaccccgccgtcgcctggccaggctgtggagtgctgccaaggggactggtgcaac
CysLysThrProProSerProGlyGlnAlaValGluCysCysGlnGlyAspTrpCysAsn
                  860                 880                 900
aggaacgtcacggcccggctgcccactaaagggaaatccttccctggatcgcagaacttc
ArgAsnValThrAlaArgLeuProThrLysGlyLysSerPheProGlySerGlnAsnPhe
                  920                 940                 960
cacctggaagttggccttatcatcctctccgtggtgtttgcggtatgccttttcgcttgc
HisLeuGluValGlyLeuIleIleLeuSerValValPheAlaValCysLeuPheAlaCys
                  980                1000                1020
atccttggcgttgctctcaggaagtttaaaaggcgcaatcaagagcgcctgaaccccaga
IleLeuGlyValAlaLeuArgLysPheLysArgArgAsnGlnGluArgLeuAsnProArg
                 1040                1060                1080
gacgtggagtacggtactatcgaagggctcatcaccaccaacgtcggagatagcactcta
AspValGluTyrGlyThrIleGluGlyLeuIleThrThrAsnValGlyAspSerThrLeu
                 1100                1120                1140
gcggaattactagatcactcgtgtacatcaggaagtggctccggtcttccttttctggta
AlaGluLeuLeuAspHisSerCysThrSerGlySerGlySerGlyLeuProPheLeuVal
                 1160                1180                1200
cagagaactgtggctcgacagataaccctgttggagtgtgtcgggaagggccggtatgga
GlnArgThrValAlaArgGlnIleThrLeuLeuGluCysValGlyLysGlyArgTyrGly
                 1220                1240                1260
gaagtgtggaggggcagctggcaaggcgaaaatgttgctgtgaagatcttctcctcccgt
GluValTrpArgGlySerTrpGlnGlyGluAsnValAlaValLysIlePheSerSerArg
                 1280                1300                1320
gatgagaagtcgtggttcagggagacagaattgtacaacacggtgatgctgaggcatgag
AspGluLysSerTrpPheArgGluThrGluLeuTyrAsnThrValMetLeuArgHisGlu
                 1340                1360                1380
aatatcttaggtttcattgcttcagacatgacctctagacactccagtacccagctgtgg
AsnIleLeuGlyPheIleAlaSerAspMetThrSerArgHisSerSerThrGlnLeuTrp
                 1400                1420                1440
ctcattacacattaccacgaaatgggatcgttgtatgactaccttcagctcaccactctg
LeuIleThrHisTyrHisGluMetGlySerLeuTyrAspTyrLeuGlnLeuThrThrLeu
                 1460                1480                1500
gacacggttagctgccttcggatcgtgttgtccatagccagcggccttgcacacttgcac
AspThrValSerCysLeuArgIleValLeuSerIleAlaSerGlyLeuAlaHisLeuHis
                 1520                1540                1560
atagagatatttgggacccaggggaagtctgccatcgcccaccgagatctaaagagcaaa
IleGluIlePheGlyThrGlnGlyLysSerAlaIleAlaHisArgAspLeuLysSerLys
                 1580                1600                1620
aacatcctcgtgaagaagaacggacagtgctgcatagcagatttgggcctggcagtcatg
AsnIleLeuValLysLysAsnGlyGlnCysCysIleAlaAspLeuGlyLeuAlaValMet
                 1640                1660                1680
cattcccagagcacgaatcagcttgatgtgggaaacaacccccgtgtggggaccaagcgc
HisSerGlnSerThrAsnGlnLeuAspValGlyAsnAsnProArgValGlyThrLysArg
```

FIG. 1 CONT.

1700 1720 1740
tacatggcccctgaagtgcttgatgaaaccatccaagtggattgctttgattcttataag
TyrMetAlaProGluValLeuAspGluThrIleGlnValAspCysPheAspSerTyrLys
1760 1780 1800
agggtcgatatttgggcctttggcctcgttctgtgggaagtggccaggaggatggtgagc
ArgValAspIleTrpAlaPheGlyLeuValLeuTrpGluValAlaArgArgMetValSer
1820 1840 1860
aatggtatagtggaagattacaagccaccattctatgatgttgttcccaatgacccaagt
AsnGlyIleValGluAspTyrLysProProPheTyrAspValValProAsnAspProSer
1880 1900 1920
tttgaagatatgaggaaagttgtctgtgtggatcaacagaggccaaacatacctaacaga
PheGluAspMetArgLysValValCysValAspGlnGlnArgProAsnIleProAsnArg
1940 1960 1980
tggttctcagacccgacattaacttctctggcgaagctgatgaaagaatgctggtaccag
TrpPheSerAspProThrLeuThrSerLeuAlaLysLeuMetLysGluCysTrpTyrGln
2000 2020 2040
aacccatccgccagactcacagctctacgtatcaaaaagactttgaccaaaattgataac
AsnProSerAlaArgLeuThrAlaLeuArgIleLysLysThrLeuThrLysIleAspAsn
2060
tccctagacaaattaaaaactgactgttga
SerLeuAspLysLeuLysThrAspCysEnd

FIG. 2

```
        10                  30                  50
atggcggagtcggccggagcctcctccttcttcccccttgttgtcctcctgctcgccggc
MetAlaGluSerAlaGlyAlaSerSerPhePheProLeuValValLeuLeuLeuAlaGly
        70                  90                 110
agtggcgggtccgggcccccgggggatccaggctctgctgtgtgcatgcaccagctgccta
SerGlyGlySerGlyProArgGlyIleGlnAlaLeuLeuCysAlaCysThrSerCysLeu
       130                 150                 170
cagaccaactacacctgcgaaacagatggggcctgcatggtctccatctttaacctggat
GlnThrAsnTyrThrCysGluThrAspGlyAlaCysMetValSerIlePheAsnLeuAsp
       190                 210                 230
ggcatggagcaccacgtacgcacctgcatccccaaggtggagcttgtgcctgctgggaag
GlyMetGluHisHisValArgThrCysIleProLysValGluLeuValProAlaGlyLys
       250                 270                 290
cccttctactgcctgagttcagaggacctgcgcaacacgcactgctgctatattgacttc
ProPheTyrCysLeuSerSerGluAspLeuArgAsnThrHisCysCysTyrIleAspPhe
       310                 330                 350
tgcaacaagattgacctgagggtgcccagtggacacctcaaggagcctgagcacccctcc
CysAsnLysIleAspLeuArgValProSerGlyHisLeuLysGluProGluHisProSer
       370                 390                 410
atgtggggccctgtggagctggtcggcatcattgccggtcctgtcttcctcctcttcctc
MetTrpGlyProValGluLeuValGlyIleIleAlaGlyProValPheLeuLeuPheLeu
       430                 450                 470
atcatcatcatcgtcttcctggtcatcaactatcatcagcgtgtctaccacaaccgccaa
IleIleIleIleValPheLeuValIleAsnTyrHisGlnArgValTyrHisAsnArgGln
       490                 510                 530
agactggacatggaggacccctcatgtgagatgtgtctctccaaagacaagacgctccag
ArgLeuAspMetGluAspProSerCysGluMetCysLeuSerLysAspLysThrLeuGln
       550                 570                 590
gatctcgtctacgatctctccacttcaggatcgggctcagggttacccctttttgtccag
AspLeuValTyrAspLeuSerThrSerGlySerGlySerGlyLeuProLeuPheValGln
       610                 630                 650
cgcacagtggcccgaaccattgttttacaagagattatcggcaagggccggtttggggaa
ArgThrValAlaArgThrIleValLeuGlnGluIleIleGlyLysGlyArgPheGlyGlu
       670                 690                 710
gtatggcgtggccgctggaggggtggtgatgtggctgtgaaaatcttctcttcccgtgaa
ValTrpArgGlyArgTrpArgGlyGlyAspValAlaValLysIlePheSerSerArgGlu
       730                 750                 770
gagcggtcgtggttccgggaggcagagatctaccagactgtcatgctgcgccatgaaaac
GluArgSerTrpPheArgGluAlaGluIleTyrGlnThrValMetLeuArgHisGluAsn
       790                 810                 830
atccttgggtttattgctgctgacaataaagacaatggcacctggacccagctgtggctt
IleLeuGlyPheIleAlaAlaAspAsnLysAspAsnGlyThrTrpThrGlnLeuTrpLeu
       850                 870                 890
gtctctgactatcacgagcacggctcactgttcgattatctgaaccgctacacagtgacc
ValSerAspTyrHisGluHisGlySerLeuPheAspTyrLeuAsnArgTyrThrValThr
       910                 930                 950
attgaggggatgattaaactggccctgtctgcagccagtggtttggcacacctgcatatg
IleGluGlyMetIleLysLeuAlaLeuSerAlaAlaSerGlyLeuAlaHisLeuHisMet
       970                 990                1010
gagattgtgggcactcaggggaagcctggaattgctcatcgagacttgaagtcaaagaac
GluIleValGlyThrGlnGlyLysProGlyIleAlaHisArgAspLeuLysSerLysAsn
      1030                1050                1070
attctggtgaagaagaatggcatgtgtgccattgcagacctgggcctagctgtccgtcac
IleLeuValLysLysAsnGlyMetCysAlaIleAlaAspLeuGlyLeuAlaValArgHis
      1090                1110                1130
gatgctgtcactgacaccatagacattgctccaaatcagagggtgggaaccaaacgatac
AspAlaValThrAspThrIleAspIleAlaProAsnGlnArgValGlyThrLysArgTyr
```

FIG. 2 CONT.

```
      1150                1170                1190
atggctcctgaagtacttgacgagaccatcaacatgaagcactttgactccttcaagtgt
MetAlaProGluValLeuAspGluThrIleAsnMetLysHisPheAspSerPheLysCys
      1210                1230                1250
gccgatatctacgccctcgggcttgtctattgggagattgctcggaggtgcaattctgga
AlaAspIleTyrAlaLeuGlyLeuValTyrTrpGluIleAlaArgArgCysAsnSerGly
      1270                1290                1310
ggagtccatgaagagtatcaactgccatattatgatttagtgccctctgacccttccatt
GlyValHisGluGluTyrGlnLeuProTyrTyrAspLeuValProSerAspProSerIle
      1330                1350                1370
gaggaaatgcgaaaggtcgtctgtgaccagaagctacggcccaatgtccccaactggtgg
GluGluMetArgLysValValCysAspGlnLysLeuArgProAsnValProAsnTrpTrp
      1390                1410                1430
cagagttatgaggccttgcgagtgatggggaagatgatgcgggagtgctggtacgccaat
GlnSerTyrGluAlaLeuArgValMetGlyLysMetMetArgGluCysTrpTyrAlaAsn
      1450                1470                1490
ggtgctgcccgcctgacagcgctgcgcatcaagaagactttgtcccagctaagcgtgcag
GlyAlaAlaArgLeuThrAlaLeuArgIleLysLysThrLeuSerGlnLeuSerValGln
      1510
gaagacgtgaagatttaa
GluAspValLysIleEnd
```

FIG. 3

```
            270                 290                 310
atgaccctggggatttttcgaagggtcttttgatgctgtcggtggccttgggcctaact
MetThrLeuGlyIlePheArgArgValPheLeuMetLeuSerValAlaLeuGlyLeuThr
            330                 350                 370
aagggagacttggtgaagccctccaggggtcagctggtaaactgcacttgtgagaaccca
LysGlyAspLeuValLysProSerArgGlyGlnLeuValAsnCysThrCysGluAsnPro
            390                 410                 430
cactgcaagaggccaatctgccaggggcatggtgcacagtggtgctagttcgagagcag
HisCysLysArgProIleCysGlnGlyAlaTrpCysThrValValLeuValArgGluGln
            450                 470                 490
ggcaggcacccccaggtctatcggggctgcgggagcctgaaccaggagctctgcctggga
GlyArgHisProGlnValTyrArgGlyCysGlySerLeuAsnGlnGluLeuCysLeuGly
            510                 530                 550
cgtcccacggagtttgtgaaccatcactgctgctatagatccttctgcaaccacaatgtg
ArgProThrGluPheValAsnHisHisCysCysTyrArgSerPheCysAsnHisAsnVal
            570                 590                 610
tccctgatgctggaggccacccaaactccttcggaggagccagaagtagatgcccatctg
SerLeuMetLeuGluAlaThrGlnThrProSerGluGluProGluValAspAlaHisLeu
            630                 650                 670
cctctgatcctgggtcccgtgctggccttgctggtcctggtggccctgggcactctgggc
ProLeuIleLeuGlyProValLeuAlaLeuLeuValLeuValAlaLeuGlyThrLeuGly
            690                 710                 730
ttgtggcgtgtccggagaaggcaggagaagcagcggggtctgcacagtgacctgggcgag
LeuTrpArgValArgArgArgGlnGluLysGlnArgGlyLeuHisSerAspLeuGlyGlu
            750                 770                 790
tccagtctcatcctgaaggcatcggaacagggagacagcatgttgggggacttcctggtc
SerSerLeuIleLeuLysAlaSerGluGlnGlyAspSerMetLeuGlyAspPheLeuVal
            810                 830                 850
agcgactgtaccacaggcagcggctcagggctacccttcttggtgcagaggacagtagcg
SerAspCysThrThrGlySerGlySerGlyLeuProPheLeuValGlnArgThrValAla
            870                 890                 910
cgacaggttgcactggtggagtgtgtgggaaagggccgatatggcgaggtgtggcgcggt
ArgGlnValAlaLeuValGluCysValGlyLysGlyArgTyrGlyGluValTrpArgGly
            930                 950                 970
tcgtggcatggcgagagtgtggcggtcaagattttctcctcacgagatgagcagtcctgg
SerTrpHisGlyGluSerValAlaValLysIlePheSerSerArgAspGluGlnSerTrp
            990                1010                1030
ttccgggagacagagatctacaacacagttctgcttagacacgacaacatcctaggcttc
PheArgGluThrGluIleTyrAsnThrValLeuLeuArgHisAspAsnIleLeuGlyPhe
           1050                1070                1090
atcgcctccgacatgacctcgcggaactccagcacgcagctgtggcttatcacccactac
IleAlaSerAspMetThrSerArgAsnSerSerThrGlnLeuTrpLeuIleThrHisTyr
           1110                1130                1150
cacgagcatggctccctctatgactttctgcagaggcagacgctggagccccagttggcc
HisGluHisGlySerLeuTyrAspPheLeuGlnArgGlnThrLeuGluProGlnLeuAla
           1170                1190                1210
ctgaggctggctgtgtccgcggcctgcgctggcctggcgcacctgcatgtagagatcttt
LeuArgLeuAlaValSerAlaAlaCysAlaGlyLeuAlaHisLeuHisValGluIlePhe
           1230                1250                1270
ggcactcaaggcaaaccagccatcgcccatcgtgacctcaagagccgcaacgtgctggtc
GlyThrGlnGlyLysProAlaIleAlaHisArgAspLeuLysSerArgAsnValLeuVal
           1290                1310                1330
aagagcaacttgcagtgttgcattgcagacctgggattggctgtgatgcactcgcaaagc
LysSerAsnLeuGlnCysCysIleAlaAspLeuGlyLeuAlaValMetHisSerGlnSer
           1350                1370                1390
agcgattacctggacattggtaacaacccccgagtgggtaccaagagatacatggcaccc
SerAspTyrLeuAspIleGlyAsnAsnProArgValGlyThrLysArgTyrMetAlaPro
```

FIG. 3 CONT.

```
                1410                1430                1450
gaggtgctggatgagcagatccgcacagactgttttgagtcgtacaagtggacagacatc
GluValLeuAspGluGlnIleArgThrAspCysPheGluSerTyrLysTrpThrAspIle
                1470                1490                1510
tgggccttcggcttagtgctatgggagattgcccggcggaccatcatcaatggcattgtg
TrpAlaPheGlyLeuValLeuTrpGluIleAlaArgArgThrIleIleAsnGlyIleVal
                1530                1550                1570
gaggactacaggccacccttctatgacatggtacccaatgaccccagttttgaggacatg
GluAspTyrArgProProPheTyrAspMetValProAsnAspProSerPheGluAspMet
                1590                1610                1630
aaaaaggtggtgtgtgttgaccagcagacccccaccatccctaaccgactggcagcagat
LysLysValValCysValAspGlnGlnThrProThrIleProAsnArgLeuAlaAlaAsp
                1650                1670                1690
ccggtcctctccgggctggcccagatgatgcgagagtgctggtaccccaacccctccgct
ProValLeuSerGlyLeuAlaGlnMetMetArgGluCysTrpTyrProAsnProSerAla
                1710                1730                1750
cgcctcaccgcactgcgcataaagaagacattacagaagctcagccagaatccagagaaa
ArgLeuThrAlaLeuArgIleLysLysThrLeuGlnLysLeuSerGlnAsnProGluLys
                1770
cccaaagtgattcactag
ProLysValIleHisEnd
```

FIG. 4

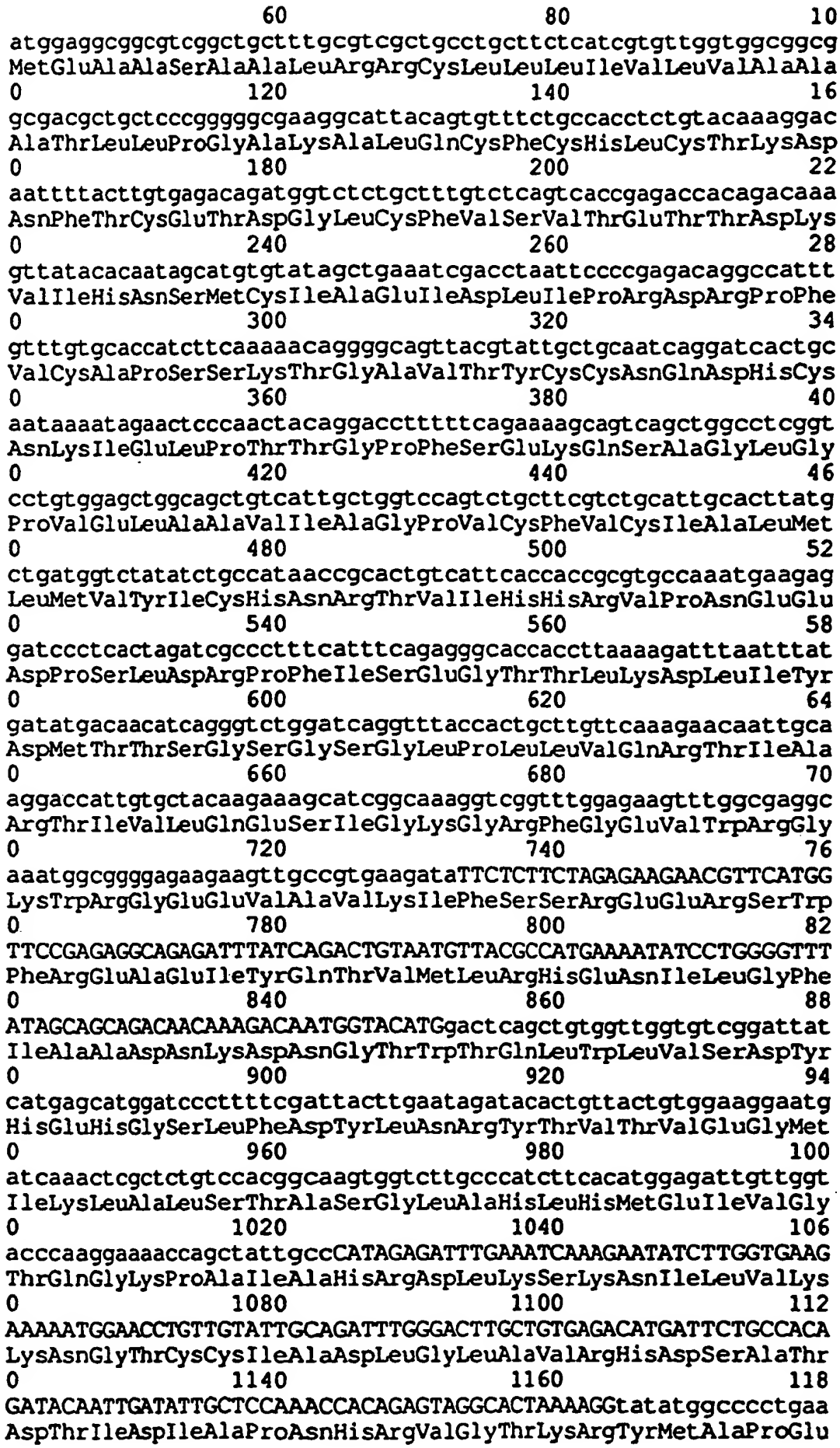
60 80 10
atggaggcggcgtcggctgctttgcgtcgctgcctgcttctcatcgtgttggtggcggcg
MetGluAlaAlaSerAlaAlaLeuArgArgCysLeuLeuLeuIleValLeuValAlaAla
0 120 140 16
gcgacgctgctcccgggggcgaaggcattacagtgtttctgccacctctgtacaaaggac
AlaThrLeuLeuProGlyAlaLysAlaLeuGlnCysPheCysHisLeuCysThrLysAsp
0 180 200 22
aattttacttgtgagacagatggtctctgctttgtctcagtcaccgagaccacagacaaa
AsnPheThrCysGluThrAspGlyLeuCysPheValSerValThrGluThrThrAspLys
0 240 260 28
gttatacacaatagcatgtgtatagctgaaatcgacctaattccccgagacaggccattt
ValIleHisAsnSerMetCysIleAlaGluIleAspLeuIleProArgAspArgProPhe
0 300 320 34
gtttgtgcaccatcttcaaaaacaggggcagttacgtattgctgcaatcaggatcactgc
ValCysAlaProSerSerLysThrGlyAlaValThrTyrCysCysAsnGlnAspHisCys
0 360 380 40
aataaaatagaactcccaactacaggacctttttcagaaaagcagtcagctggcctcggt
AsnLysIleGluLeuProThrThrGlyProPheSerGluLysGlnSerAlaGlyLeuGly
0 420 440 46
cctgtggagctggcagctgtcattgctggtccagtctgcttcgtctgcattgcacttatg
ProValGluLeuAlaAlaValIleAlaGlyProValCysPheValCysIleAlaLeuMet
0 480 500 52
ctgatggtctatatctgccataaccgcactgtcattcaccaccgcgtgccaaatgaagag
LeuMetValTyrIleCysHisAsnArgThrValIleHisHisArgValProAsnGluGlu
0 540 560 58
gatccctcactagatcgccctttcatttcagagggcaccaccttaaaagatttaatttat
AspProSerLeuAspArgProPheIleSerGluGlyThrThrLeuLysAspLeuIleTyr
0 600 620 64
gatatgacaacatcagggtctggatcaggtttaccactgcttgttcaaagaacaattgca
AspMetThrThrSerGlySerGlySerGlyLeuProLeuLeuValGlnArgThrIleAla
0 660 680 70
aggaccattgtgctacaagaaagcatcggcaaaggtcggtttggagaagtttggcgaggc
ArgThrIleValLeuGlnGluSerIleGlyLysGlyArgPheGlyGluValTrpArgGly
0 720 740 76
aaatggcggggagaagaagttgccgtgaagataTTCTCTTCTAGAGAAGAACGTTCATGG
LysTrpArgGlyGluGluValAlaValLysIlePheSerSerArgGluGluArgSerTrp
0 780 800 82
TTCCGAGAGGCAGAGATTTATCAGACTGTAATGTTACGCCATGAAAATATCCTGGGGTTT
PheArgGluAlaGluIleTyrGlnThrValMetLeuArgHisGluAsnIleLeuGlyPhe
0 840 860 88
ATAGCAGCAGACAACAAAGACAATGGTACATGgactcagctgtggttggtgtcggattat
IleAlaAlaAspAsnLysAspAsnGlyThrTrpThrGlnLeuTrpLeuValSerAspTyr
0 900 920 94
catgagcatggatccctttcgattacttgaatagatacactgttactgtggaaggaatg
HisGluHisGlySerLeuPheAspTyrLeuAsnArgTyrThrValThrValGluGlyMet
0 960 980 100
atcaaactcgctctgtccacggcaagtggtcttgcccatcttcacatggagattgttggt
IleLysLeuAlaLeuSerThrAlaSerGlyLeuAlaHisLeuHisMetGluIleValGly
0 1020 1040 106
acccaaggaaaaccagctattgccCATAGAGATTTGAAATCAAAGAATATCTTGGTGAAG
ThrGlnGlyLysProAlaIleAlaHisArgAspLeuLysSerLysAsnIleLeuValLys
0 1080 1100 112
AAAAATGGAACCTGTTGTATTGCAGATTTGGGACTTGCTGTGAGACATGATTCTGCCACA
LysAsnGlyThrCysCysIleAlaAspLeuGlyLeuAlaValArgHisAspSerAlaThr
0 1140 1160 118
GATACAATTGATATTGCTCCAAACCACAGAGTAGGCACTAAAAGGtatatggcccctgaa
AspThrIleAspIleAlaProAsnHisArgValGlyThrLysArgTyrMetAlaProGlu

FIG. 4 CONT.

```
0                  1200                  1220                  124
gttctagatgattccataaatatgaaacattttgaatccttcaaacgtgctgacatctat
ValLeuAspAspSerIleAsnMetLysHisPheGluSerPheLysArgAlaAspIleTyr
0                  1260                  1280                  130
gcaatgggcttagtattctgggaaatcgctcgacgctgttccattggcggaatccacgaa
AlaMetGlyLeuValPheTrpGluIleAlaArgArgCysSerIleGlyGlyIleHisGlu
0                  1320                  1340                  136
gactaccagttgccttactatgatcttgtaccttctgatccatccgttgaagaaatgaga
AspTyrGlnLeuProTyrTyrAspLeuValProSerAspProSerValGluGluMetArg
0                  1380                  1400                  142
aaagtagtttgtgaacagaagttaaggccaaatattcccaacagatggcagagctgtgag
LysValValCysGluGlnLysLeuArgProAsnIleProAsnArgTrpGlnSerCysGlu
0                  1440                  1460                  148
gccttgagagtgatggccaaaattatgagagaatgttggtatgccaatggagcagctagg
AlaLeuArgValMetAlaLysIleMetArgGluCysTrpTyrAlaAsnGlyAlaAlaArg
0                  1500                  1520                  154
ctgacagctttgcgaattaaaaaaacattgtcacagctcagccaacaggaaggcatcaaa
LeuThrAlaLeuArgIleLysLysThrLeuSerGlnLeuSerGlnGlnGluGlyIleLys
0
atgtaa
MetEnd
```

A. MISr1 (BCORI insert size -2.7 kb, specific oligo sequence: 5′-GTCTACCAGAAGGGCTGCTT-3′) (SEQ ID NO: 5)
All inserts are in the ECORI site of plasmid pBluescript I SK(-).

B. MISr2a (~1.4 kb, 5′-CCGGAGCCTCCTCCTTCTTC-3′) (SEQ ID NO: 6)

C. MISr2b (~2.1 kb, 5′-TCCCTACTGGGTTTGAGACA-3′) (SEQ ID NO: 7)

D. MISr3 (~3.2 kb, 5′-GCTGCGGGAGCCTGAACCAG-3′) (SEQ ID NO: 8)

E. MISr4 (~2.8 kb, 5′-AAATCCAATGTTTGAATACT-3′) (SEQ ID NO: 9)

FIG. 5

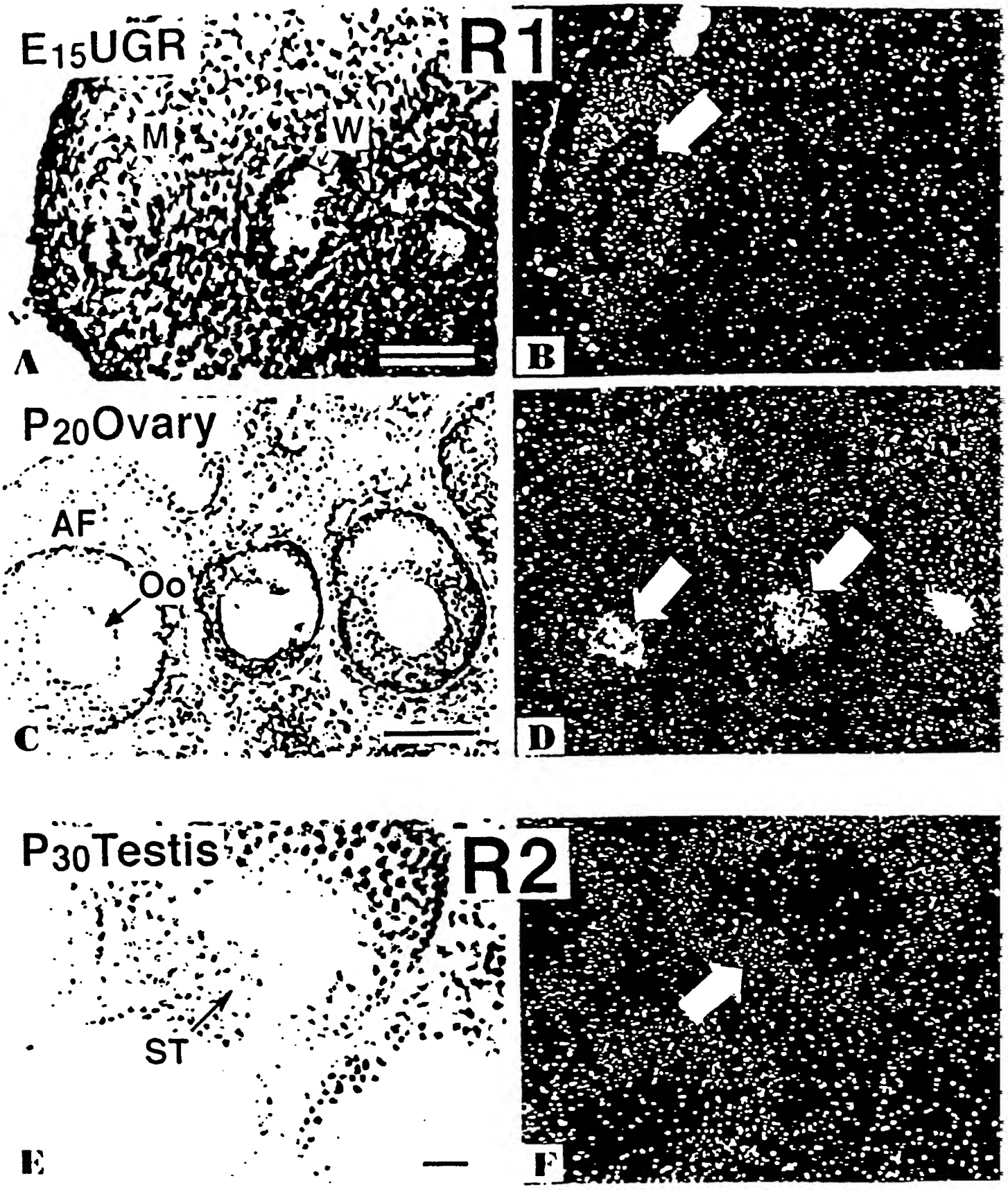

FIG. 6

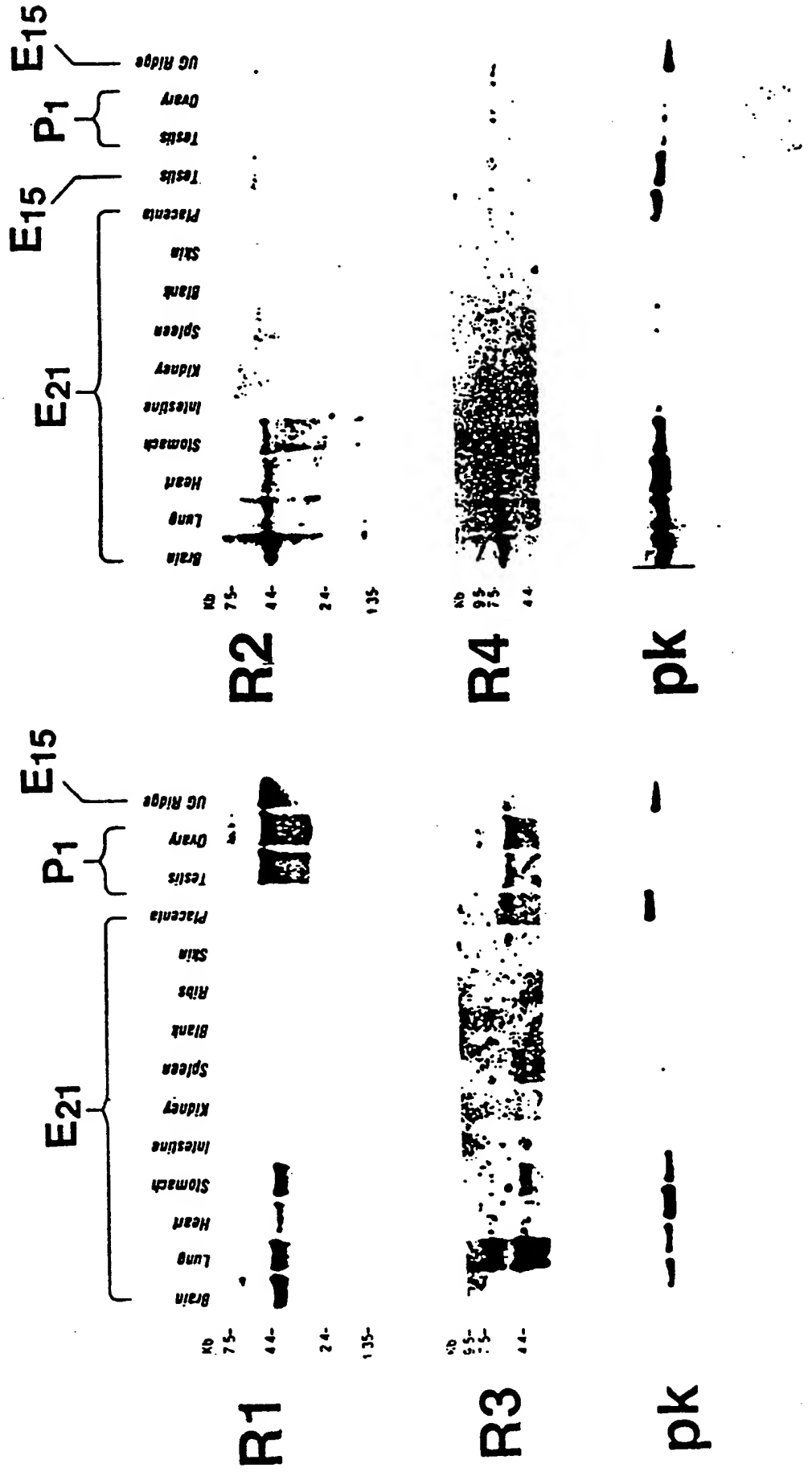
R1
R2
R3
R4
pk
pk
E21
E15
P1
E15
Brain
Lung
Heart
Stomach
Intestine
Kidney
Spleen
Blank
Ribs
Skin
Placenta
Testis
Ovary
UG Ridge
kD
75-
44-
24-
135-
55-
95-

FIG. 7

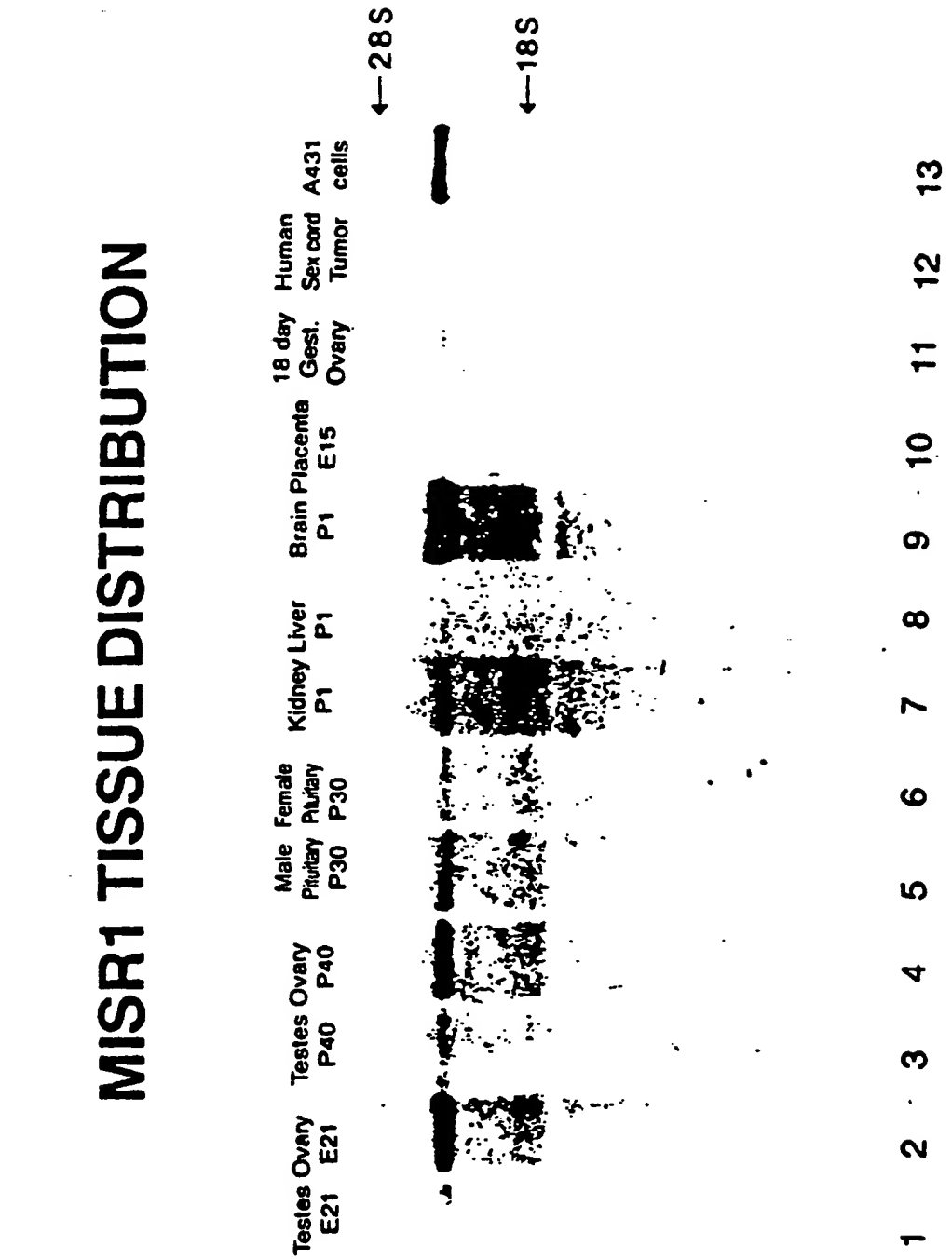
MISR1 TISSUE DISTRIBUTION
Testes E21
Ovary E21
Testes P40
Ovary P40
Male Pituitary P30
Female Pituitary P30
Kidney P1
Liver P1
Brain P1
Placenta E15
18 day Gest. Ovary
Human Sex cord Tumor
A431 cells
←28S
←18S
1
2
3
4
5
6
7
8
9
10
11
12
13

Figure 8

MISR2 TESTES ONTOGENY

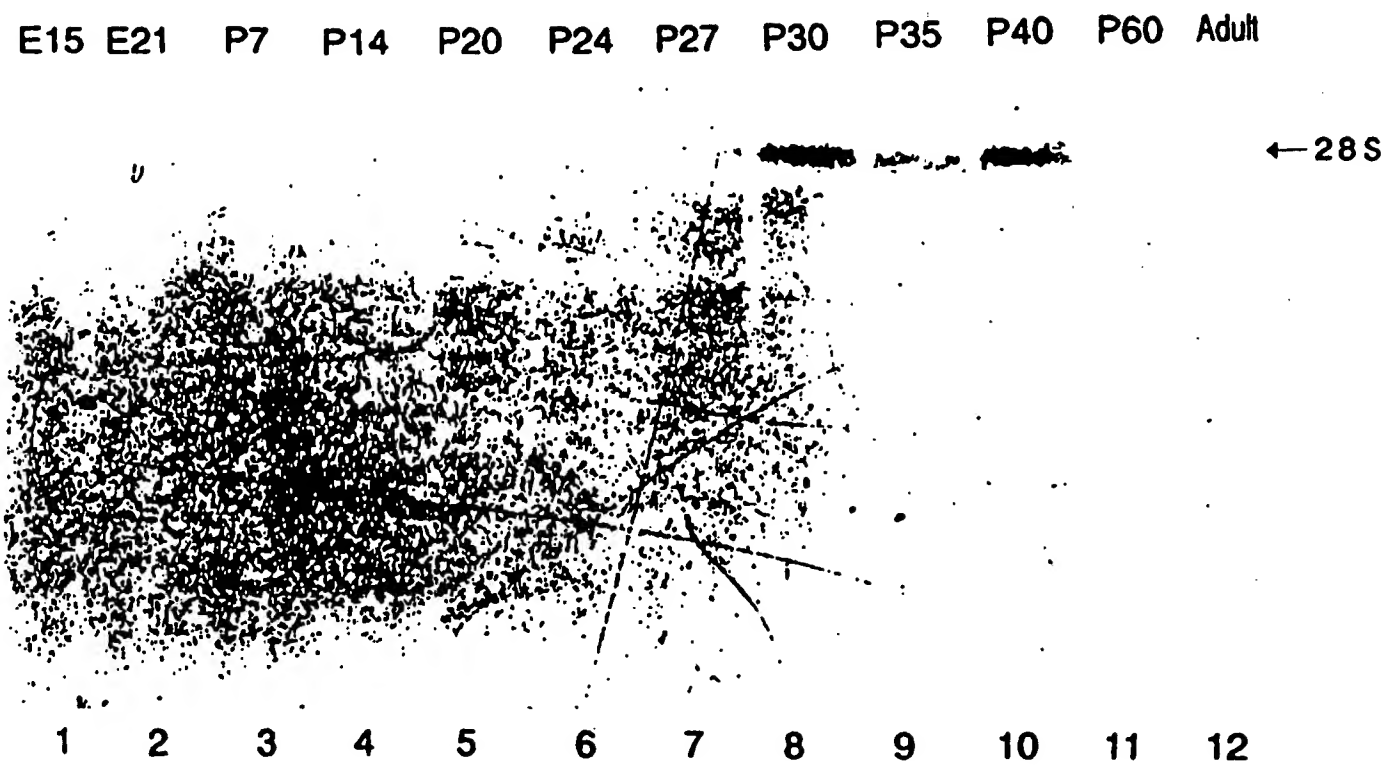

Figure 9

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 15/12, 1/00, 15/63; C07K 13/00, 17/02; C12Q 1/68, 1/00

US CL :536/23.5, 24.31; 435/69.1, 320.1, 240.1, 6, 7.1, 7.2; 530/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5, 24.31; 435/69.1, 320.1, 240.1, 6, 7.1, 7.2; 530/395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Molecular and Cellular Endocrinology, Volume 62, issued 1989, G. Lefevre et al., "Anti-idiotypic antibodies to a monoclonal antibody raised against anti-Mullerian hormone exhibit anti-Mullerian biological activity", pages 125-133, especially the abstract.	1-19, 24, 25
Y	Proceedings of the National Academy of Sciences of the USA, Volume 80, issued March 1983, R. A. Young et al., "Efficient isolation of genes by using antibody probes", pages 1194-98, especially the abstract.	1-19, 24, 25

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 07 June 1993	Date of mailing of the international search report 14 JUN 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer DAVID L. FITZGERALD Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Cell Biology, Volume 107, Number 6, part 3, C. S. Teng et al., "Identification of the Mullerian Inhibiting Substance (MIS) Receptor on the Human Tumor Cells", page 71A, abstract no. 381.	1-19, 24, 25
Y	US, A, 4,792,601 (Donahoe et al.), 20 December 1988, especially col. 16, lines 31-34	1-19, 24-25
X Y	Cell, Volume 65, issued 14 June 1991, L. S. Mathews et al., "Expression Cloning of an Activin Receptor, a Predicted Transmembrane Serine Kinase", pages 973-982, especially pages 973 and 979 and Figures 3 and 7 (the misr1 sequence exhibits 52% overall similarity to that of Fig.3 of the reference, with regions of locally higher sequence identity).	7 1-6, 7-19, 24, 25
Y	Proceedings of the National Academy of Sciences of the USA, Volume 78, Number 11, S. V. Suggs et al., "Use of synthetic oligonucleotides as hybridization probes: Isolation of cloned cDNA sequences for human ß2-microglobulin", pages 6613-6617, especially the abstract.	1-19, 24, 25
Y,P	GenBank record no. L02911, entered 29 September 1992, K. Matsuzaki et al., "Nobel serine-kinase receptor type 1" (the listed sequence exhibits 88% sequence identity to misr1).	1-19, 24, 25

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

KEYWORD DATABASES: US PTO-APS, Medline, Pascal, CancerLit, Biosis, Derwent Biotech. Abstracts, Derwent WPI
SEARCH TERMS: Mullerian Inhibit?; Bind?, Ligand, Receptor; Inhibin; Transforming growth factor beta
SEQUENCE DATABASES: GenBank, GeneSeq, EMBL, SwissProt, PIR

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-19, 24, and 25, drawn to the misr1 clone, vectors, cells, probes, peptides, and hybridization and ligand binding assays, classified in U.S. Class 536, subclasses 23.2 and 24.31; Class 435, subclasses 320.1, 240.2, 252.3, 6, and 7.6; and Class 530, subclasses 395 and 402.

II. Claims 20-23 and 26-28, drawn to MIS receptor antibodies, immunotoxins, and immunoassays, classified in U.S. Class 530, subclasses 387.1 and 391.7, and Class 435, subclass 7.21.

III. Claims 29-42 and 45, drawn to the misr2 clone, vectors, cells, probes, peptides, and hybridization and ligand binding assays, classified in U.S. Class 536, subclasses 23.2 and 24.31; Class 435, subclasses 320.1, 240.2, 252.3, 6, and 7.6; and Class 530, subclasses 395 and 402.

IV. Claims 43, 44, and 46, drawn to inhibin receptor antibodies, immunotoxins, and immunoassays, classified in U.S. Class 530, subclasses 387.1 and 391.7, and Class 435, subclass 7.21.

V. Claims 47-52, drawn to the misr3 clone, probes, and peptides, classified in U.S. Class 536, subclasses 23.2 and 24.31, and and Class 530, subclass 395.

VI. Claims 53-58, drawn to the misr4 clone, probes, and peptides, classified in U.S. Class 536, subclasses 23.2 and 24.31, and and Class 530, subclass 395.

The groups are held to lack unity of invention as follows.

The special technical feature of each group is the composition of matter, one of the clones misr1, misr2, misr3, or misr4, the MIS receptor, or the inhibin receptor. The four misr clones are disclosed as unique and distinct species which are not expected to have substantially similar properties in vivo; each thus defines a unique contribution over the prior art. While misr1 is postulated to encode a MIS receptor and misr2, an inhibin receptor, the assignments are putative only, and antibodies which recognize these receptors are known in the art. The two clones misr1 and misr2 thus define contributions over the prior art, and unity of invention does not exist between the clones and the receptors they are postulated to encode.